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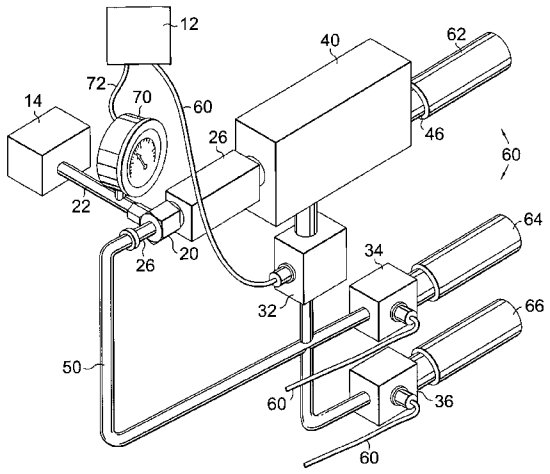
(54) Title: VORTEX TUBE THERMOCYCLER



(57) Abstract: A thermal cycling apparatus (10) utilizes hot and cold gas streams produced from pressurized gas being passed through a Ranque-Hilsch Vortex Tube (20) to efficiently and rapidly cycle samples (70) (i.e., DNA+Primer+Polymerse) between the denaturation, annealing, and elongation temperatures of the PCR process. The samples (70) are disposed within a reaction chamber (40) that, through connection with a vortex tube (20), allows the gas to contact the samples (70). The temperature of the gas that is allowed to contact the samples (70) is controlled by a valving system (30) being connected with the vortex tube (20) and the reaction chamber (40). The valving system (30) controls the flow of cold gas into the reaction chamber (40) where it is mixed with the hot gas to establish the different temperatures required for the denaturation, annealing, and elongation steps of the cycle.

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VORTEX TUBE THERMOCYCLER

FIELD OF THE INVENTION

The present invention relates generally to the field of Polymerase Chain Reaction (PCR) amplification of DNA, and, more particularly, is directed to a method and apparatus for performing a PCR process utilizing a single pressurized gas source.

BACKGROUND OF THE INVENTION

The Polymerase Chain Reaction (PCR) method of DNA amplification is a powerful and sensitive enzymatic technique used to exponentially increase the number of copies of a specific sequence of template DNA. PCR is one of the most widely used techniques in molecular biology, *See* Mullis, K. B., U.S. Patent No. 4,683,202, which is herein incorporated by reference in its entirety; Saiki, R. K., Scharf, S., Faloona, F., Mullis, D. B., Horn, G. T., Erlich, H.A., and Arnheim, N., 1985, "*Enzymatic amplification of beta-globin genomic sequences and retriction site analysis for diagnosis of sickle cell anemia,*" Science, 230, pp. 1350-1354, which is herein incorporated by reference in its entirety; Erlich, H. A., ed., 1989, "*PCR Technology: Principles and Applications for DNA Amplification,*" Stockton Press, New York, which is herein incorporated by reference in its entirety; Mullis, K., Ferre, F., and Gibbs, R. A., 1994, "*The Polymerase Chain Reaction,*" Birkhauser, Boston, which is herein incorporated by reference in its entirety. PCR-amplified DNA may be used in medical diagnostics, such as to diagnose mutations responsible for human genetic diseases (Saiki *et al.,* 1985; Kogan, S.C., Doherty, M., and Gitschier, J., 1987, "*An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences,*" New Eng. J. Med., 317, pp. 985-990, which is herein incorporated by reference in its entirety) in blood and tissue typing (Saiki, R. K., Walsh, P. S., Levenson, P. H., and Erlich, H. A., 1989, "*Genetic analysis of amplified DNA with sequence-specific oligonucleotide probes,*" Proc. Nat. Acad. Sciences USA, 86, pp. 6230-6234, which is herein incorporated by reference in its entirety), forensics, or to detect pathogens responsible for important infectious diseases (Persing, D. H., Smith, T. F., Tenover, F. C., and White, T. J., eds., 1993, "*Diagnostic Molecular Biology: Principles and Applications,*" ASM Press, Washington, D.C., which is herein incorporated by reference in its entirety; Nicoll, S., Brass, A., and Cubie, H. A., 2001, "*Detection of herpes*

viruses in clinical samples using real-time PCR," J. Virol. Methods, 96, pp. 25-31, which is herein incorporated by reference in its entirety).

The PCR process normally includes three steps: (I) Sample Preparation, (II) DNA Amplification, and (III) Detection. In step (I) a sample mixture is prepared typically consisting of a DNA sample, a primer, and a polymerase. The sample mixture is allotted into a desirable sample size (typically 1 to 100μL) and placed in an appropriate container (i.e., standard cuvette) for amplification. In step (II), DNA amplification is achieved through performance of a cycle normally consisting of three sequential steps: (1) double-stranded DNA is denatured to a single-stranded form at a high temperature (90° to 95°C), (2) the resulting single-stranded DNA strands are annealed to oligonucleotide primers at approximately 50° to 65°C, and (3) primer template complexes are elongated using a thermostable DNA Polymerase such as *Thermus aquaticus (Taq)* Polymerase at approximately 72°C (Saiki, R. K., 1989, "*The design and optimization of the PCR,*" In: *PCR Technology: Principles and Applications for DNA Amplification*, H. A. Erlich, ed., Stockton Press, New York, pp. 17-22, which is herein incorporated by reference in its entirety). The amplification step consists of thermally cycling the sample for N cycles (approximately 30-40) between the denaturing, annealing, and elongating steps. Theoretically, one cycle of these three steps (denaturation/annealing/elongation) results in a two-fold amplification of the DNA fragment whose 5' and 3' ends are defined by sequence-specific annealing of the oligonucleotide primers to the DNA template. In a typical PCR reaction, template DNA sequences lying between the ends of two defined oligonucleotide primers can be amplified in approximately 2 hours.

The DNA amplification yield of the PCR process can be described in terms of average efficiency (Y), where $X= (1+Y)^N$. An efficiency of 70% is considered satisfactory and approximately 35 cycles are required for 10^8-fold amplification. In a perfectly efficient PCR process, the cycles may give 2^{30}-fold (approximately 10^9-fold) amplification of a particular DNA sequence. Gel electrophoresis is the standard for product (DNA fragment) detection and typically employed for step (III) of the PCR process, elongation. However, real-time detection can be achieved by measuring the fluorescence of dye/DNA complexes throughout the amplification stage of the reaction. This drastically reduces total assay time by eliminating the need for an individual step for product detection. Real-Time PCR also provides the required optical measurements used in quantitative PCR analysis.

A variety of PCR machines have been built for use in practical applications which automate the three-step PCR amplification process (Oste, C. C., 1989, "*PCR Automation,*" In: Erlich H. A., ed., *PCR Technology: Principles and Applications for DNA Amplification*, Stockton Press, New York, pp. 23-30, which is herein incorporated by reference in its entirety; Oste, C. C., 1994, "*PCR Instrumentation: Where Do We Stand?,*" In: K. Mullis, F. Ferré, and R. A. Gibbs, eds., 1994, *The Polymerase Chain Reaction*, Birkhauser, Boston, pp.165-173, which is herein incorporated by reference in its entirety; Newton, C. R., 1995, "*PCR Instruments,*" In: C. R. Newton, *Essential PCR Data*, John Wiley & Sons, Chichester, England, pp.12-23, which is herein incorporated by reference in its entirety; Johnson, B., 1998, "*The Competition Heats Up. The annual review of thermal cyclers takes a sneak peak at the new products for 1998,*" The Scientist, 12, p. (24), which is herein incorporated by reference in its entirety). These devices generally may be classified into two categories: robotic devices, which move the DNA samples to the heat; and thermocyclers, which bring the heat to the samples. Robotic devices, such as Stratagene's ROBOCYCLER™ (Strategene, La Jolla, CA), move tubes containing PCR reaction samples to and from a series of water baths, which are heated to different temperatures. Although robotic thermocyclers are useful in certain research applications, they are incapable of high-speed PCR. Typically, the robotic devices require greater than 60 minutes for the performance of 30 cycles of the DNA Amplification step of the PCR process. This is the equivalent to greater than 2 minutes per complete PCR cycle (preparation/amplification/detection).

The two basic types of thermocyclers are programmable heat blocks and forced hot-air thermocyclers. In commonly employed heat block thermocyclers, the amplification stage consists of cycling the temperature of the samples using computer-controlled heat blocks. The samples, typically contained in thin-walled plastic reaction tubes (e.g., cuvettes), are inserted into holes formed in the heat block.

The problem with these devices is that they typically require hours of operation time due to the slow heating and cooling processes. Many commonly employed PCR protocols spend one minute at 94°C (DNA denaturation), one minute at approximately 55° to 60°C (primer annealing), and one minute at 72°C (elongation). For example, in the original PCR method used by Cetus workers (Saiki, R. K., 1989, "*The design and optimization of the PCR,*" In: *PCR Technology: Principles and Applications for DNA Amplification*, H. A. Erlich, ed.,

Stockton Press, New York, pp. 17-22, which is herein incorporated by reference in its entirety), a 536 b.p. human ß-globin DNA fragment was amplified using 30 cycles of (1 min at 94°C, 1 min at 55°C, 1 min at 72°C) in a total PCR time of approximately 2.5 hours. The active duty time for this thermocycling protocol is only approximately 3.5 minutes = 210 seconds. This time is the amount needed to enzymatically copy a 536 b.p. template 30 times at a *Taq* DNA Polymerase elongation rate of approximately 80 nt/sec (Innis, M. A., Myambo, K. B., Gelfand, D. H., and Brow, M. A. D., 1988, "*DNA sequencing with Thermus aquaticus DNA polymerase and direct sequencing of polymerase chain-reaction amplified DNA,*" Proc. Nat. Acad. Sciences USA 85, pp. 9436-9440, which is herein incorporated by reference in its entirety; Gelfand, D. H., and White, T. J., 1990, "*Thermostable DNA Polymerases,*" In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds., *PCR Protocols: a Guide to Methods and Applications*, Academic Press, Inc., San Diego, which is herein incorporated by reference in its entirety). Commercially available heat block thermocyclers (Perkin-Elmer, Ericomp, MJ Research, Eppendorf, Techne, BioRad, Snark Technologies) require 20 to 25 seconds in order to cool from 94°C down to 55°C; and 14 to 20 seconds to heat from 55°C to 94°C, *see* Johnson, B., 1998, "*The Competition Heats Up. The annual review of thermal cyclers takes a sneak peak at the new products for 1998,*" The Scientist, 12, p. 24, which is herein incorporated by reference in its entirety. Therefore, the "dead time" for each PCR cycle is another 40 $\pm$ 5 seconds per cycle. As shown in FIG. 1, commonly employed PCR protocols require (220 seconds/cycle x 30 cycles) = 6600 seconds = 110 minutes. *See* Saiki, R. K., 1989, "*The design and optimization of the PCR,*" In: *PCR Technology: Principles and Applications for DNA Amplification*, H. A. Erlich, ed., Stockton Press, New York, pp. 17-22, which is herein incorporated by reference in its entirety. Only approximately 3.5 minutes of the 110 minutes is productively focused on the PCR process.

Forced hot air thermocyclers, such as the Rapid Cycler™ (Idaho Technology, USA) and the LightCycler™ (Roche Diagnostics Corporation, Germany) have drastically reduced typical amplification time by eliminating the large thermal mass of heat blocks and utilizing convection heat transfer between air and thin-walled capillary tubes. PCR Amplification consisting of 30 cycles can be performed in as little as approximately 10 to 30 minutes utilizing these forced hot air thermocyclers. The reduction in time for performance of one PCR Amplification cycle of denaturing/annealing/elongating is the

result of: (1) heat transfer from forced hot air to the aqueous biochemical reaction sample was carried out in thin-walled capillary tubes; (2) the reaction chamber had very low thermal mass; and (3) the denaturation and annealing holding times during the PCR cycle were minimized. The rate-limiting biochemical step in the three-step PCR reaction sequence (denaturation/ annealing/elongation) is the rate of DNA Polymerase elongation. At an elongation rate of approximately 80 nucleotides/sec by *Taq* Polymerase (Innis, M. A., Myambo, K. B., Gelfand, D. H., and Brow, M. A. D., 1988, "*DNA sequencing with Thermus aquaticus DNA polymerase and direct sequencing of polymerase chain-reaction amplified DNA,*" Proc. Nat. Acad. Sciences USA 85, pp. 9436-9440, which is herein incorporated by reference in its entirety; Gelfand, D. H., and White, T. J., 1990, "*Thermostable DNA Polymerases,*" In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds., *PCR Protocols: a Guide to Methods and Applications*, Academic Press, Inc., San Diego, which is herein incorporated by reference in its entirety), theoretically one second per cycle is needed to amplify DNA fragments ≤ 100 b.p. using approximately 20mer primers. For example, only about 5 seconds per cycle were needed to enzymatically copy a 536 b.p. ß-globin amplicon using *Taq* Pol (Idaho Technology, 1995; cf. Fig. 1b). Using 10 μL volumes and a PCR protocol of 30 cycles of [0 sec 94°C (denaturation), 0 sec 55°C (annealing), 5 sec 72°C (elongation)], a 536 b.p. ß-globin DNA fragment was amplified in 9.9 minutes; or 19.8 seconds/cycle (Idaho Technology, 1995). The hot-air thermocycling protocol was approximately 220/19.8 = 11 times faster than the original method of Saiki et al., (1989), *See* Saiki, R. K., 1989, "*The design and optimization of the PCR,*" In: *PCR Technology: Principles and Applications for DNA Amplification*, H. A. Erlich, ed., Stockton Press, New York, pp. 17-22, which is herein incorporated by reference in its entirety, for PCR amplification of a 536 b.p. ß-globin DNA fragment using a conventional heat block thermocycler.

In practice, very few researchers use 10µL PCR reactions; and the process is considerably slower. Thus, in routine laboratory diagnostic PCR, the hot-air thermocycler is not as fast as described above. For example, Nicoll, S., Brass, A., and Cubie, H. A., 2001, "*Detection of herpes viruses in clinical samples using real-time PCR,*" J. Virol. Methods, 96, pp. 25-31, which is herein incorporated by reference in its entirety, amplified short (96 to 220 b.p.) long herpes virus DNA fragments through 50 PCR cycles of [0 sec 94°C (denaturation), 3 sec 58° to 67°C (annealing), 6 sec 72°C (elongation)] in approximately 25

minutes; or about 30 seconds/cycle using the Roche LightCycler™. The design of forced hot-air thermocyclers, *see* Wittwer, C. T., Hillyard, D. R., and Ririe, K. M., 1987, "*Rapid thermal cycling device,*" U.S. Patent No. 5,455,175, which is herein incorporated by reference in its entirety; Wittwer, C. T., Filmore, G. C., and Garling, D. J., 1990, "*Minimizing the time required for DNA amplification by efficient heat transfer to small samples,*" Anal. Biochem. 186, pp. 328-331, which is herein incorporated by reference in its entirety; Wittwer, C.T., Reed, G. B., and Ririe, K. M., 1994, "*Rapid Cycle DNA Amplification,*" In: Mullis, K., Ferré, F., and R. A. Gibbs, eds., *The Polymerase Chain Reaction,* Birkhauser, Boston, pp.174-181, which is herein incorporated by reference in its entirety; Zurek, T. F., Hanley, K. A., and Pepe, C. J., 1996, "*Method for thermal cycling nucleic acid assays,*" U.S. Patent No. 5,576,218, which is herein incorporated by reference in its entirety, is an improvement over block heaters. However, forced hot-air thermocyclers operate at atmospheric pressure, using fans or "blowers" for gas mixing. Therefore the velocities achieved are not very high (<3 m/sec) and relatively low convective heat transfer rates result, as compared to what is possible with high velocity turbulent gas flow. Neither, Wittwer et al., (1987; 1990; 1994) or Zurek et al. (1996) have used gases other than air, pressures other than atmospheric, cooling gases colder than 22°C, or time constants faster than one second. Further, flow conditioners have not been used to mix hot and cold gases.

The heat transfer conditions needed for high-speed amplification of DNA using hot/cold pressurized gas bursts are most accurately termed turbulent, non-isothermal compressible gas flow, *See* Welty, J. R., Wicks, C. E., and Wilson, R. E., 1976, "*Fundamentals of Momentum, Heat, and Mass Transfer*", Second Edition, John Wiley & Sons, New York, which is herein incorporated by reference in its entirety; and virtually none of the optimal gas parameters or flow conditions has previously been utilized.

PCRJet™ (owned by Megabase Research Products, Lincoln, Nebraska) operates at gas pressures, velocities, turbulent non-isothermal flow conditions and geometries that have not been considered previously. FIG. 1 provides a schematic comparison of temperature vs. time profiles of various Thermocyclers for Polymerase Chain (PCR) amplification of DNA. The major differences between a hot-air thermocycler and a pressurized gas jet thermocycler are summarized in Table I below.

Table I: Comparison of Hot-air Thermocycler to Pressurized Gas Jet Thermocycler

Properties	*A. Hot-air Thermocycler*	*B. Pressurized Gas Thermocycler*
Hot Gas	Air	Air, N_2, or He
Cold Gas	Air @ 23°C	Air or CO_2 @ <20°C
Pressure	P = 1 atm	$P \geq 3$ atm
Gas Velocity	$V < 3$ m/sec	$5 < V < 30$ m/sec
Chamber Configuration	Heat Chamber = Rxn Chamber	Heat Chamber ≠ Rxn Chamber
Number of Valves	1	≥ 2
Type of Valve	Butterfly	Electronic
Gas Mixing	Fan	Flow Conditioner
Rate-limiting Step	Machine Dead Time	DNA Pol Elongation
Time for 1 PCR Cycle	>14 sec/cycle	≤2.6 sec/cycle
Time for 30 PCR Cycles (200 to 500 b.p. amplicons)	10 to 60 minutes	1.5 to 6.0 minutes
Control Software	BASIC™, LabView™	VisualBasic™, PID Control
Programmable Time Interval	1 second	≤ 0.1 seconds

In engineering terms, the differences between the atmospheric hot-air thermocycler and the pressurized gas jet thermocycler are best described by a brief analysis of heat transfer around a sample container/thin cylinder (capillary). The cylinder is chosen for this section because both of these thermocyclers use capillaries for holding the DNA samples; but the analysis would be analogous for any other shape of sample holder. Let d_o and d_i denote the outer and inner diameters of the capillary tube. Natural convection heat transfer and internal thermal gradients in the reacting liquid mixture are negligible due to the dominant role of viscous forces and small inner diameter. A simplified thermal energy balance (neglecting conduction in the fluid) of the aqueous solution inside the capillary is given by

$$\rho_m Cp_m \frac{dT_s}{dt} = \frac{4}{d_i} h_{eff} (T_g - T_s), \quad (1)$$

where ρ_m and Cp_m denote the mixture's density and specific heat capacity. This equation describes how fast the temperature of the sample in the capillary (denoted by T_s) changes when a gas at temperature T_g flows over the capillary. For example, suppose the sample is at 94°C and it must be cooled down to 56°C by flowing cold gas at 0°C over the capillary.

Setting the time $t = 0$ when cold gas starts to flow, the time needed for the aqueous sample inside the capillary to cool down to can be obtained by solving equation (1):

$$t = -\frac{\rho_m Cp_m d_i}{4 h_{eff}} \ln\left[\frac{273 + 56}{273 + 94}\right] = \frac{0.03 \rho_m Cp_m d_i}{h_{eff}}$$

(2)

In a similar manner, any heating or cooling of biochemical reaction samples can be described. It is evident from equation (2) that the time t is reduced when the effective heat transfer coefficient h_{eff} is maximized. This coefficient links the performance of the thermal cycler to the heat transfer and the fluid mechanics. Under conditions used in the PCRJet™, h_{eff} is determined by heat transfer through the boundary layer around the capillary and thus it is affected by the fluid mechanics. Very fast heating and cooling of DNA reaction samples inside capillary tubes is possible if this boundary layer is thin; i.e., under conditions of turbulent flow.

FIG. 2 shows the output, on a computer screen, for a PCRJet™. Utilizing advanced gas dynamics and improved heat transfer properties the PCRJet™ completed thirty cycles in 42 seconds. FIG. 3 shows the DNA fingerprint of the amplification of a 368 b.p. *Bacillus anthracis* DNA fragment by the PCRJet™. As indicated in lane [e] of FIG. 3, the approximate 10^8-fold amplification of a 368 base pair diagnostic *Bacillus anthracis* DNA fragment was achieved using 30 PCR cycles in three minutes and fifty-three seconds (233 seconds) or less than 4 minutes.

The PCRJet™ thermocycler has set speed records for every DNA amplicon tested. In general, viral and bacterial DNA fragments 100 to 500 b.p. long were typically amplified through 30 PCR cycles in 2 to 4 minutes; whereas single copy human gene fragments in this size range required approximately 3.5 to 6.0 minutes for 35 PCR cycles. A short list of reaction times for 30 to 35 high-speed PCR cycles, starting with 10 picograms of DNA, is listed in Table II. DNA amplification experiments are organized into 4 areas: [1] High-Speed Amplification of Bacterial DNAs, [2] PCR-based Diagnosis of Heritable Human Diseases, [3] Long PCR, [4] Nested PCR. Reaction yields were typically 30 to 100 ng of amplified DNA (greater than 10^8-fold amplification), corresponding to an average efficiency per cycle of 79% to 87%.

Table II: Summary of PCRJet™ DNA Amplification Experiments

[1] ***High-Speed Amplification of Bacterial DNA:***	***(b.p.)***	***(Min:Sec)***	*(seconds)*
E.coli O157:H7 (*Stx2* gene fragment)	**85 bp**:		
30 cycles x [0 sec 86C, 0 sec 63C, 0 sec 72C] =		**1:18**	(78 seconds)
E.coli uidA (β-glucuronidase gene fragment)	**145 bp**:		
33 cycles x [0 sec 87C, 0 sec 66C, 0.4 sec 72C] =		**2:06**	(144 seconds)
E.coli uidA (β-glucuronidase gene fragment)	**297 bp**:		
30 cycles x [0 sec 87C, 0 sec 58C, 0.8 sec 72C] =		**2:13**	(153 seconds)
E.coli uidA (β-glucuronidase gene fragment)	**486 bp**:		
30 cycles x [0.1 sec 89C, 0.1 sec 65C, 1.5 sec 72C] =		**3:01**	(181 seconds)
Bacillus anthracis Sterne strain amplicon	**368 bp**:		
30 cycles x [0 sec 92C, 0 sec 55C, 3.2 sec 72C] =		**3:53**	(233 seconds)
E.coli rrnA (conserved 16S rRNA) amplicon	**150 bp**:		
35 cycles x [0.2 sec 90C, 0.2 sec 56C, 2.5 sec 72C] =		**4:40**	(280 seconds)
Bacillus cereus amplicon	**600 bp**:		
30 cycles x [0 sec 90C, 0 sec 57C, 2.5 sec 72C] =		**4:53**	(293 seconds)
[2] *High-Speed Amplification of Single Copy Human Gene Fragments:*			
Human ABO 'blood type A-transferase	**147 bp**:		
35 cycles x [0.2sec 94C, 0.2 sec 55C, 0.9 sec 72C] =		**3:50**	(230 seconds)
Human Dihydropyrimidine Dehydrogenase	**198 bp**:		
35 cycles x [0.2 sec 94C, 0.2 sec 57C, 1.0 sec 72C] =		**3:27**	(207 seconds)
Human Platelet Antigen HPA-4 allele	**364 bp**:		
35 cycles x [0 sec 92C, 0 sec 55C, 5.0 sec 72C] =		**5:35**	(335 seconds)
Human β-Globin	**536 bp**:		
35 cycles x [0.5 sec 94C, 0.5 sec 55C, 4,0 sec 72C] =		**6:02**	(362 seconds)

[3] ***Long PCR Experiments:***

Bacteriophage λ **2206 bp**:

30 cycles x [0.5 sec 91C, 0.5 sec 61C, 8.0 sec 72C] = **8:53** (453 seconds)

Bacteriophage λ **2331 bp**:

30 cycles x [0.5 sec 91C, 0.5 sec 61C, 13 sec 72C] = **10:17** (617 seconds)

[4] ***Nested PCR Experiment:***

E.coli uidA (β-glucuronidase) **486/186 bp**:

20 cycles x [0.2 sec 88C, 0.2 sec 56C, 2.8 sec 72C] +

20 cycles x [0.2 sec 88C, 0.2 sec 65C, 1.8 sec 72C] **4:45** (285 seconds)

The PCRJet™ has shown to be superior to other thermocyclers with respect to speed while operating within acceptable tolerances for thermal control (+/- 0.2°C). It also improves upon ease-of-use via the PC user interface. As shown in Fig. 2, the PCRJet™ is capable of carrying out 30 cycles of [0 sec 90°C, 0 sec 58°C] in as little as 42 seconds; or 1.4 seconds/cycle.

The experimental thermocyclers built by Lawrence Livermore National Laboratory (Northrup, M. A., Benett, B., Hadley, D., Landre, P., Lehew, S., Richards, J., and Stratton, P., 1998, "*A miniature analytical instrument for nucleic acids based on micromachined silicon reaction chambers*", Anal. Chem., 70, pp. 918-922, which is herein incorporated by reference in its entirety), the University of Pittsburgh (Oda, R. P., Strausbauch, M. A., Huhmer, A. F., Jurrens, S. R., Craighead, J., Wettstein, P. J., Eckloff, B., Kline, B., and Landers, J. P., 1998, "*Infrared-mediated thermocycling for ultrafast polymerase chain reaction* amplification *of DNA*", Anal. Chem., 70, pp. 4361-4368, which is herein incorporated by reference in its entirety), the University of Washington (Friedman, N. A., and Meldrum, D. R., 1998, "*Capillary tube resistive thermal cycling*", Anal. Chem., 70, pp. 2997-3002, which is herein incorporated by reference in its entirety), and the Massachusetts Institute of Technology (Chiou, J., Matsudaira, P., Sonin, A., and Ehrlich, D., 2001, "*A Closed-cycle Capillary Polymerase Chain Reaction Machine*", Anal. Chem., 73, pp. 2018-2021, which is herein incorporated by reference in its entirety) required 8.5 to 23 minutes for 30 PCR cycles; or 17 to 46 sec/cycle. The PCRJet™ results, shown in

Fig.2 require less than 1.4 seconds/ cycle to cover a thermal gradient of 32°C (90°C to 57°C to 90°C).

The PCRJet™ thermocycler, however, uses a pair of high wattage (W) process heaters to heat air to 180°C and a heat exchanger (ice bath) for cooling. Heaters are known to be prone to failure and the device is not considered mobile enough for multiple point-of-care sites.

The heating and cooling performance is the primary concern in all of the automated thermocyclers. It would be desirable to provide a thermal cycling device that provides both the heating and cooling necessary for the thermal cycling of a sample from a single source. Further, it would be desirable to provide a thermal cycling device which is capable of employing a variety of gases, pressures other than and including atmospheric, utilizes faster time constants, cools gases colder than 22°C, and optimizes fluid mechanics. Additionally, it would be desirable to provide a thermal cycling device that is mobile enough to be utilized at multiple point-of-care sites.

The thermocycler of the present invention described below uses the natural heating and refrigeration capacities of the Ranque-Hilsch vortex tube shown in FIG. 4. A vortex tube is a device that produces two gas streams of significant temperature difference from a single source of compressed gas. An attractive feature of the vortex tube is its reliability. It operates without any moving parts or electrical components. Also, it is small, lending itself to be part of a compact system.

In order to produce the two separated gas streams, high-pressure air (typically 2-11 atm) enters a cylindrical container tangentially through one or more ports. After entering the cylinder, the gas spirals down the length of the tube where it meets a control valve. According to the valve setting, a fraction of the gas (x) will exit the tube at an outlet temperature (T_H) up to about 110°C warmer than the inlet temperature of the gas. The remaining gas fraction (1-x) exits at a temperature (T_C), which has a maximum temperature difference of about 70°C lower than the initial gas temperature. The outlet temperatures can be readily varied according to the absolute pressure drops across the tube, the type of gas used, and with the control valve that changes the fraction of gas exiting at each end. FIG. 5 illustrates vortex tube operation for one set of flow conditions.

SUMMARY OF THE INVENTION

The present invention provides a thermal cycling apparatus that uses the natural heating and refrigeration capacities of a Ranque-Hilsch Vortex Tube to provide the heating and cooling necessary for the performance of the PCR process. The Ranque-Hilsch Vortex Tube produces two streams of gas having significant temperature differences from a single source of compressed gas. The fraction of the gas that exhausts out the hot end exhaust may exit the tube at an outlet temperature of up to approximately one hundred ten (110°C) degrees Celsius warmer than the inlet temperature of the gas. Further, the fraction of the gas that exhausts out the cold end exhaust may exit the tube at an outlet temperature of up to approximately seventy (70°C) degrees Celsius colder than the inlet temperature of the gas. It is understood, that the outlet temperature of the gas exhausting from the hot end exhaust and/or the cold end exhaust may be greater than the values previously described without departing from the scope and spirit of the present invention. The gas pressure for entering the Ranque-Hilsch Vortex Tube may typically range from two to eleven atmospheres. Alternatively, the gas pressure may be less than 2 atmospheres or greater than eleven atmospheres.

For high-speed amplification of DNA using hot and cold pressurized gas bursts, the heat transfer conditions needed are termed turbulent, non-isothermal compressible gas flow. The present invention provides heat transfer conditions that are turbulent, non-isothermal compressible gas flow, through the use of the Ranque-Hilsch Vortex Tube connected with control valves and a reaction chamber. The result is a significant decrease in the amount of time required for the performance of a PCR cycle. For example, the present invention may perform a single PCR cycle in less than or equal to 10 seconds and allow for the proper biochemical reactions to occur.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed. The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate an embodiment of the invention and together with the general description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The numerous advantages of the present invention may be better understood by those skilled in the art by reference to the accompanying figures in which:

FIG. 1 is a schematic representation comparison of Temperature vs. Time profiles of various Thermocyclers for Polymerase Chain (PCR) amplification of DNA .

FIG. 2 is a representation of a Temperature vs. Time (seconds) output for thermo-cycling utilizing the PCRJet;

FIG. 3 is a photographic representation of a DNA fingerprint from the high-speed amplification of 368 b.p. Bacillus anthracis DNA fragment utilizing the PCRJet. 10 pg of DNA from B. anthracis Sterne strain were amplified in a 16 ul PCR reaction in a 1 mm OD glass capillary tube containing 1.25 U KOD HotStart DNA Pol (Toyobo Co. Ltd), 1 uM 21mer oligonucleotide primers, 3.5 mM Mg++, and 500 ug/ml BSA. After PCR, DNA fragments were separated on a 2% agarose gel and stained using 0.7 ug/ml EtBr. M.W. markers are in lane [a]. 30 cycles of PCR were carried out using: [b] [0 sec 92C, 0 sec 55C, 4.2 sec 72C], [c] [0 sec 92C, 0 sec 55C, 3.8 sec 72C], [d] [0 sec 92C, 0 sec 55C, 3.5 sec 72C], [e] [0 sec 92C, 0 sec 55C, 3.2 sec 72C]. In lane [e] the total time needed for 10^8-fold amplification of the 368bp B. anthracis amplicon was 3 minutes, 53 seconds (233 seconds). High-speed amplification of 368 b.p. Bacillus anthracis DNA fragment. 10 pg of DNA from B. anthracis Sterne strain were amplified in a 16 ul PCR reaction in a 1 mm OD glass capillary tube containing 1.25 U KOD HotStart DNA Pol (Toyobo Co. Ltd), 1 uM 21mer oligonucleotide primers, 3.5 mM Mg++, and 500 ug/ml BSA. After PCR, DNA fragments were separated on a 2% agarose gel and stained using 0.7 ug/ml EtBr. M.W. markers are in lane [a]. 30 cycles of PCR were carried out using: [b] [0 sec 92C, 0 sec 55C, 4.2 sec 72C], [c] [0 sec 92C, 0 sec 55C, 3.8 sec 72C], [d] [0 sec 92C, 0 sec 55C, 3.5 sec 72C], [e] [0 sec 92C, 0 sec 55C, 3.2 sec 72C]. In lane [e] the total time needed for 10^8-fold amplification of the 368bp B. anthracis amplicon was 3 minutes, 53 seconds (233 seconds).

FIG. 4 is an illustration of a prior art Ranque-Hilsch vortex tube.

FIG. 5 is an illustration of the Ranque-Hilsch Vortex Tube showing one set of flow conditions.

FIG. 6 is perspective view of a thermal cycling apparatus in accordance with an exemplary embodiment of the present invention.

FIG. 7 is a diagrammatic illustration of an exemplary embodiment of the thermal cycling apparatus of the present invention configured with a flow pattern for sample heating.

FIG. 8 is a diagrammatic illustration the thermal cycling apparatus configured with a flow pattern for sample cooling.

FIG. 9 is a phase diagram illustrating typical temperature deviations within a reaction chamber of the thermal cycling apparatus of the present invention and differences in temperature change rates between heating and cooling processes;

FIG 10 is perspective view of the reaction chamber of the present invention with a view of the sample capillaries.

FIG. 11 is perspective view of an exemplary embodiment of the mixing chamber of the present invention.

FIG. 12 is a perspective view of an exemplary sample chamber of the thermal cycling apparatus.

FIG. 13 is a diagrammatic illustration of the optical detection system in an exemplary embodiment of the present invention.

FIG. 14 is a diagrammatic illustration of an alternative embodiment of the optical detection system in an exemplary embodiment of the present invention.

FIG. 15 is a diagrammatic illustration of an exemplary embodiment of the thermal cycling apparatus having a supplemental heating device associated with the "hot" end of the vortex tube.

FIG. 16 is a phase diagram illustrating a temperature vs. time profile for a hot start and initial ten cycles of a PCR reaction being performed by the thermal cycling apparatus.

FIG. 17 is a photographic image depicting a DNA fingerprint of the high-speed amplification of 0 to 100 pg of 96 bp λ-DNA Amplicon performed utilizing the thermal cycling apparatus of the present invention.

FIG. 18 is phase diagram depicting real-time PCR spectrometer data corresponding with the high-speed amplification of 0 to 100 pg of 96 bp λ-DNA Amplicon;

FIG. 19 is a table displaying successful PCR results from the thermal cycling apparatus of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to the presently preferred embodiments of the invention, examples of which are illustrated in the accompanying drawings. The drawings illustrate a Polymerase Chain Reaction (PCR) thermocycler. The thermocycler utilizes hot and cold gas streams produced from pressurized gas being passed through a Ranque-Hilsch Vortex Tube to efficiently and rapidly cycle samples (i.e., DNA+Primers+Polymerase+dNTPs) between the denaturation, annealing, and elongation temperatures of the PCR process. The compact design and limited dependence on electricity allows this machine to operate as a portable device, which may be advantageously suited for field use.

Referring generally now to FIGS. 6 through 8, an exemplary embodiment of the thermocycler of the present invention is generally designated at 10. The thermocycler includes a vortex tube 20 having an inlet 22, a hot gas outlet 24 and a cold gas outlet 26. The hot gas outlet 24 is in fluid communication with the reaction chamber 40 and the cold gas outlet 26 is in fluid communication with a gas flow conduit 50. The gas flow conduit is further connected to a valving system, generally indicated at 30, which preferably comprises three electronically actuated pneumatic valves that direct the cold gas flow as further explained hereinafter. The apparatus 10 also includes a processor 12, which can be programmed to cause the reaction chamber 40 to be cycled through a series of temperatures over a predetermined period of time. The thermocycler of the present invention thus can be used to carry out numerous procedures and potential applications may include transplantation surgery, diagnosis of heritable and infectious disease agents, point-of-care diagnostic testing, biological warfare agent detection, virology, microbiology, hematology, pharmaco-genetics, forensics, and oncology research.

The valving system 30 directs the cold gas from the gas flow conduit 50 to either the reaction chamber 40 or exhausts the cold gas. The valving system comprises a first valve, or cold flow valve 32, which connects with the reaction chamber 40 to selectively allow cold gas to be directed to the reaction chamber and the samples N held therein, when necessary. The remaining two valves are cold release valves 34, 36 that exhaust cold gas out of the system during heating processes. Preferably, a means for sound attenuation, indicated generally at 60 is also provided. This means 60 includes a first muffler device 62,

which connects with the reaction chamber 40, and second and third muffler devices 64, 66 which connect with the cold release valves, 34 and 36.

The reaction chamber 40 is divided into a mixing chamber 42 and a sample chamber 44. The thermal cycling apparatus 10 optimizes the convective heat transfer to one or more sample cuvettes, preferably six 20 μL sample capillaries, 70 held in the sample chamber during both the heating and cooling processes. The temperatures of the PCR samples are indirectly measured via a thermal sensor placed in an additional capillary tube. This signal is continually utilized by processor 12, which in turn controls the valving system 30 to direct the cold air flow within the system according to the sample temperature change required.

The thermal cycling apparatus 10 heats the samples by allowing only the hot gas to pass through the sample chamber 44, as shown in FIG. 7. In this scenario, the cold flow valve 32 is closed and the two cold release valves 34 are open. This valve configuration provides two exhausts for the cold gas produced by the vortex tube to exit the system with negligible back pressure. Meanwhile, the gas exiting the hot end of the vortex tube flows across the samples located in the sample chamber prior to exiting the system via the sample chamber exhaust 46. The vortex tube 20 is adjusted as is well known in the art such that the hot gas temperature is approximately 105°C and the corresponding volumetric flow rate of hot gas produced is approximately 6.4×10^{-4} m^3/s (1.4 SCFM). With these parameters, the thermal cycling apparatus 10 can heat the samples at an average rate of approximately 3.2°C/sec.

The transition from sample heating to sample cooling must be performed without interrupting the operating vortex tube by stopping flow on the cold side. Therefore, the cold flow valve 32 is opened prior to closing the cold release valves 34 and 36. Adequate cooling is achieved under this configuration (FIG. 8) where all of the cold and hot gases mix in the mixing chamber 42 before reaching the sample chamber 44. Since the mass flow rate of cold gas is about four times greater than the mass flow rate of the hot gas, the resulting stream cools the samples in the sample chamber 44 to the annealing temperature. The cold gas is approximately -5°C exiting the vortex tube and the cold/hot gas mixture in the mixing chamber 42 is typically around 25°C. The samples are cooled at an average rate of about 16°C/sec. As will be understood by those skilled in the art, alternative

cooling configurations are also possible by varying the two release valves between open and closed positions while the cold flow valve remains open.

Many rapid PCR protocols include temperature holds at the denaturation, annealing, or elongation temperatures. These are achieved by the processor 12 via computer-controlled (cold gas) valve programming for the specified time durations. The thermal cycling apparatus 10 allows hot gas to continually flow into the sample chamber, so fine cool air control is used to neutralize its effect on the samples and minimize temperature fluctuations during holds. The fine control is achieved by opening the primary cold release valve 34, the secondary cold release valve 36, or a combination of the two, while varying the cold flow valve 32 between the open and closed positions. Under these configurations there is always cold gas exiting the system, however, a fraction of the cold gas is occasionally routed into the chamber to provide near isothermal sample conditions. The programming logic for the processor 12 uses set temperature limits and temperature vs. time slope measurements to control the status of the cold flow valve 32. The typical temperature deviation within the chamber during these holds is shown in FIG. 9. When there are no holds required at the denaturation or annealing temperatures, overshooting and undershooting is minimized through feedback control from the previous thermal cycle. This enables the thermal cycling apparatus 10 to make heating and cooling transition adjustments within a PCR run to maximize performance by accurately reaching the desired target temperatures. FIG. 9 also shows the difference in temperature change rates between heating and cooling process as described earlier.

The thermal cycling apparatus 10 of the present invention is capable of operating as a portable device. Under an exemplary embodiment, its compact design of the thermocycler gives it overall dimensions of approximately 12 inches by 8 inches by 6 inches. It is to be understood that the dimensions of the thermocycler may vary to increase portability and on-site application capabilities. For example, the current embodiment could be redesigned to a smaller overall dimension by switching to more custom components. The thermal cycling apparatus 10 also has very limited dependence on electricity and can be controlled using a personal laptop computer as the processor 12.

The general operation of the thermal cycling apparatus 10 is described in the preceding paragraphs. The following sections offer a detailed description of some of the individual components of the thermocycler that are critical to its operation.

Ranque-Hilsch Vortex Tube

The basis for the thermal cycling device is the vortex tube 20 (Exair Corporation, Cincinnati, OH). The vortex tube is most commonly used in industry for cooling applications where the exit streams are at atmospheric pressure. The thermal cycling apparatus 10 of the present invention uses the vortex tube within a system to provide both the hot and cold gas streams necessary for the thermal cycling of the samples. Since the device is entirely dependent on the performance of the vortex tube, its operational parameters are optimized.

The first step in optimizing the vortex tube 20 is to size the generator, a flow restriction device, to correspond with the available air supply. The appropriate generator should maximize the volumetric flow rate, while providing enough resistance to create a large pressure drop across the tube 20. The tube 20 operates most efficiently at inlet pressures of about 5.5 – 7.5 bar (~80-110 psi). In the preferred embodiment, the compressed gas supply used by the thermal cycling apparatus 10 is capable of delivering room temperature air at a flow rate of approximately 3.2×10^{-3} m^3/sec (6.8 SCFM) at a gage pressure of approximately 5.9 bar (85 psi). Other inlet flow conditions are possible without deviating from the scope of the present invention.

This is a standard condition used for all of the performance results stated in this document. If the inlet pressure is decreased, the mass flow rate and the temperature of the hot gas exiting the vortex tube will decrease along with thermocycler performance. Eventually, if it is reduced to a temperature below typical denaturation temperatures the thermocycler will be incapable of supporting PCR. If a compressor or bottled gas is being used that can supply inlet air at a higher pressure or flow rate, the performance of the thermocycler could be increased. However, this will alter design parameters for this particular device and may effect its normal operation (i.e. large temperature gradients may become present between sample capillaries along flow direction). New machine or chamber configurations could be implemented to take advantage of this increased performance that would negate any

negative side effects observed under the current setup. This would be done without deviating from the concepts stated for the current invention.

The thermal cycling apparatus 10 typically employs compressed air, as the source 14, supplied to the gas inlet 22 of the vortex tube 20 by a standard air compressor. It is to be understood that alternate gases, such as helium, may be used. Helium provides thermal conductivity characteristics across a helium boundary layer that may increase the heat transfer. This is advantageous for the present invention as heat transfer (hot or cold) is a critical performance characteristic of the PCR process.

The vortex tube 20 is most effective when the exit streams are near atmospheric pressure. The performance of the vortex tube 20 decreases dramatically with the addition of back pressure on the cold side. For the vortex tube 20 used in the thermal cycling apparatus 10 of the present invention, the cold gas temperature will increase by approximately 2.8°C with 0.345 bar (5 psi) of back pressure. Accordingly, the hot gas temperature will decrease with increasing back pressure on the cold side. This is important for the thermal cycling apparatus 10 since it is using the vortex tube within a system where back pressure is present and the primary factor in its cycling performance is the temperature of the hot air leaving the vortex tube. Therefore, the amount of back pressure is minimized with the appropriate fittings and valve orifices. Under normal operation, the back pressure on the cold side of the vortex tube should not exceed 3 psi.

The exit temperatures are variable according to the ratio of gas exiting at each end of the vortex tube, 24 and 26. Typically, the exit temperature difference at each end is inversely related to the mass fraction of gas exiting. For this application, it has been concluded from an experimental investigation that the hot gas temperature must exceed 100°C to ensure the sample reaches the denaturation temperature after experiencing minimal heat loss throughout the system. To obtain the minimum increase in hot gas temperature of 80°C, the vortex tube 20 must be set so the fraction of hot gas exiting is low, approximately 20% of the inlet flow. The resulting cold flow exits the vortex tube at less than 0°C. Accordingly, the tube becomes more effective during the cooling process and sample heating becomes the rate-limiting step.

Preferably, an insulation chamber 26 is provided around the hot end 24 of the vortex tube 20 to maximize vortex tube performance. There is significant heat loss from the hot gas to the tube walls. This corresponds to high speed flow along a plate, *see* Kays, W. and Crawford, M, 1993, *Convective heat and mass transfer,* Third Edition. McGraw-Hill, Inc. New York, which is present in the vortex tube from a highly tangential velocity component. The insulation material placed around the hot end 24 of the vortex tube 20 must not only have beneficial insulating properties, but must also be able to withstand temperatures up to 150°C. Melamine foam may be used as the insulating material, which is rated to approximately 200°C, but other materials could be used without deviating from the scope of the invention. Ultimately, the insulation chamber used in this thermocycler increases the hot gas temperature by 9.7°C and decreases the time for 30 cycles [0 sec 90°C, 0 sec 56°C, 0 sec 72°C] by 43 seconds.

Electronically Controlled Pneumatic Valves

The thermal cycling apparatus 10 is controlled by a computer through the use of an I/O card. With this machine/computer interface the computer is able to receive the analog signals from the thermocouples and inlet pressure gauge and send digital signals to control the status of the three solenoid valves of the valving system 30. The programming logic is specifically written for the machine in Visual Basic (Microsoft, Redmond, WA) and provides the user interface for operation. To perform a PCR run, the user inputs a specific PCR protocol and then hits the start button. The large window displays the real-time temperature readings from the sample chamber while reaction progress is shown in terms of cycle number, cycle time, total time, and percent complete in the PCR status window. The control logic also enables the user to store reaction data (temperature vs. time and spectrometer data) in electronic file format after completing a reaction.

In the current embodiment, all the valves are electronically actuated, direct acting, 24 VDC, normally closed, 2-way valves with a complete cycle time of approximately 16-30 msec. Each 2-way valve mechanism operates identically in that there is both an "open" and "closed" position. When "open," gas is allowed to flow through the valve while no gas flow occurs when the valve is in the "closed" position. The amount of cold gas that is directed into the mixing chamber 42 of the reaction chamber 40 can be varied according to the status of the three valves. For example, the configuration shown in FIG. 8 directs all of

the cold gas into the chamber 42. But by opening one or both of the release valves 34, 36 some of the cold gas will be exhausted, which ultimately decreases the amount of cold gas being directed into the sample chamber 44. It is to be understood that other flow configurations are possible using variable valves instead of these standard "open" vs. "closed" 2-way valves and that these configurations are within the scope of the present invention.

The valves of valve system 30 are preferably composed of stainless steel to offer structural integrity while minimizing thermal conductivity. It is contemplated that the valves may be composed of various other metals, composite materials, and the like. The use of various metals, composite materials, and the like may assist in decreasing flow resistance through the valves and operational stresses, such as thermal stresses and vibration. The valve orifice sizes can also be modified according to the flow rate of gas being used and the desired pressure drop across the valves. In the current embodiment, relatively large 6mm orifice sizes are used to minimize resistance to flow through the valves.

The cold flow valve 32 and first and second cold release valves 34, 36, respectively, are electronically actuated through a link 60 to the processor or controller 12. In a preferred embodiment, the processor 12 is an information handling system, such as a computer, which is capable of communicating commands for the operation of the valves. The processor is enabled to execute a set of instructions (a.k.a., software program/control logic/programming logic) for the performance of the PCR process by the thermal cycling apparatus 10. The instruction set includes commands for the operation of the valve system 30 during the PCR process. Further, the instruction set may control the flow of gas into the vortex tube 20 from the gas supply via a link between the controller device and the gas supply. Under this configuration, the gas is supplied to the vortex tube via a gauge 70 for tracking the volume of gas being supplied and the pressure of the gas. The controller device 12 may be electronically linked through link 72 to the gauge 70, which may have a control flow valve. The controller device 12 may execute commands from the instruction set which are transmitted to the gauge whereby the control flow valve is able to be set in various positions, such as open and closed, to control the flow of gas into the vortex tube.

Reaction Chamber

In a preferred embodiment, the reaction chamber is a housing comprising a vortex flow conditioner or mixing chamber 42, and a sample chamber 44. The mixing chamber 42 is in fluid communication, via a first inlet conduit 46, with the hot end 24 of the vortex tube 20 and with the cold flow valve 32 via a second inlet conduit 48.

The first step in creating near isothermal reaction conditions in the sample chamber 44 is to deliver well-mixed and uniformly distributed air flow. This is important in the thermal cycling apparatus 10 since it sends hot and cold gas streams into the mixing chamber 42 simultaneously during the cooling stroke and the annealing-elongation-denaturation temperature holding periods. Therefore, the mixing chamber 42 is required to effectively mix two inlet gas streams, G1 and G2, respectively, of significant temperature difference to an equilibrium temperature prior to the gas stream entering the sample chamber 44. This mixing is achieved through a swirling flow field induced by the tangentially oriented inlet 48 of the cold gas stream into a cylindrical mixing chamber 42, as shown in FIG. 11.

The mixing chamber 42 preferably used in the thermal cycling apparatus 10 is shown in FIG. 11. Preferably, both the hot and cold gas streams enter the cylindrical cavity (diameter = 1 in) through 7/16 inch diameter ports, but only the cold gas inlet 48 enters tangentially. As previously described, there is no gas mixing during the heating processes. The hot gas exits the vortex tube and flows generally straight through the mixing chamber 42 into the sample chamber 44 relatively undisturbed. When mixing is required, the cold gas enters perpendicular to the hot gas inlet 46, overwhelms it, and induces a swirling motion. Important consideration is given to the design of the mixing chamber 42 in its ability to mix the hot and cold gas streams, but it also must not impede cycling performance. Preferably, the mixing chamber is cylindrical, as shown in FIG. 11, which is beneficial in that it has very low thermal mass (product of density and heat capacity) and low thermal conductivity with the use of a balsa wood liner. This reduces heat loss or gain to or from the surrounding walls during each cycle, which ultimately increases performance. More intrusive designs, such as baffles, and flow distribution and mixing elements, may be used and are within the scope of the present invention.

The configuration of the mixing chamber 42 may be varied further to accommodate different volumes of gas. The vortex flow conditioner 42 is in fluid communication with

the sample chamber 44 via a flow conduit 47, allowing the mixed hot and cold gas steam to pass into the sample chamber. It is to be understood that the first and second inlet conduits 46 and 48, respectively, and the flow conduit 47 may be variously configured to maximize the efficient utilization of the non-isothermal, turbulent, three-dimensional gas flow being used by the thermal cycling apparatus 10. The configuration of the conduits and mixing chamber 42 may be established relative to one another to assist in maximizing the efficiency of the vortex flow conditioner in mixing the hot and cold gases.

The sample chamber 44 likewise has an important role in creating consistent reaction conditions across the samples. Capillary 70 placement and channel dimensions for gas flow are important factors that significantly affect the thermal variance across samples and cycling performance. In a preferred embodiment, the sample chamber 44 includes a series of ducts, indicated generally at 80. As shown in FIG. 13, these ducts are recessed into the housing 44 and exposed on one end to the outside environment. The ducts 80 may recess various distances within the housing as contemplated by those of ordinary skill in the art. The sample chamber includes a flow conduit 82, which extends through the chamber 44 and is in contact with the ducts. The sample chamber flow conduit 82 provides for the flow of gas to come into contact with the ducts 80 and anything received within the ducts, such as sample cuvettes C. It is to be understood that the sample chamber flow conduit 82 is configured in order to maximize the flow pattern of the gas by and around the ducts. As described previously, the gas flow is a non-isothermal, turbulent, three-dimensional flow and as such the sample chamber flow conduit 82 is constructed to maximize the heat transfer from the gas flow, G, to the sample containers 70.

As the gas flows through the reaction chamber 44, heat is exchanged with the conduit walls 84 (wall effect) inside the reaction chamber 44 and the capillaries 70. In order to assist in minimizing the difference in heat transfer to the capillaries 70, between the first capillary and subsequent capillaries, heat exchange with the conduit walls 84 of the reaction chamber 44 must be minimized. In a preferred embodiment, the material selected for the conduit walls 84 of the reaction chamber has a low thermal conductivity and low specific heat capacity. Additionally, the material is thermally stable to gas temperatures of at least 130°C. It is to be understood that the wall effect may be reduced by placing the capillaries 70 close together. However, there is a limit on how close the capillaries 70 may be placed

since upstream capillaries (e.g., 70A, 70B) cause flow disturbances that affect heat transfer of downstream capillaries (e.g., 70H, 70I).

In the current embodiment, the sample chamber 44 is designed to accommodate the thin-walled glass capillaries 70 typically used in the thermal cycling apparatus 10. The capillaries 70 have a 1.5 mm outer diameter, total length of 3 cm, and are designed for sample volumes ranging from 5 to 20 μL. Other sample containers can be used without deviating from the scope of the invention.

As shown in FIG. 12, the sample chamber conduit walls 84 are preferably tapered to narrow from inlet to outlet to reduce the variance between samples at the denaturation temperature and to allow for more useable sample positions. As the gas flows through each conduit 82, its velocity will increase as the channel width decreases. This increase in gas velocity increases the heat transfer capability from the gas to the sample capillary 70 to compensate for the shadowing and wall effects increasingly experienced from the front to back sample positions. Preferably, the chamber 44 has a total of 9 positions: 6 for PCR samples, 1 for thermocouple control, and 2 "dummy" positions located at the front of the chamber 44. The staggered arrangement in the chamber is designed to minimize the affects of preceding capillaries shadowing subsequent ones. However, it is clear that the first two capillaries heat and cool much faster since they do not experience any shadowing. Therefore, positions 1-2 are not used for samples, but are present to aid in flow distribution for subsequent capillaries.

The axial spacing of the samples 70 is minimized according to the size restriction of the capillary head 72 and the transverse staggering. The capillaries are positioned parallel with the tapered conduit walls 84 to create the staggered arrangement and so any wall effects will be consistent. The inlet 86 of the conduit 82 has a width of about 6.4 mm (0.251 in) and corresponding gas velocities of 5.6 m/s during sample heating and 28.2 m/s for the maximum cooling configuration. By the time the gas reaches the final capillary, the conduit width has decreased to about 2.8 mm (0.110 in). The gas velocity exiting the chamber is approximately 12.9 m/s during sample heating and 64.4 m/s for the maximum cooling configuration. The channel height for the conduit 82 must remain a constant 18 mm (0.7 in) to accommodate the 20 μL sample volume.

It is to be understood that the number of ducts 80 included within the sample chamber 44 may vary without departing from the scope and spirit of the present invention. In the current embodiment, the ducts 80 are arranged in a staggered pattern. This staggered arrangement assists in reducing temperature differential effects the first capillary has on subsequent capillaries, the second capillary has on subsequent capillaries, and so forth. It is contemplated that the dimensional characteristics of this staggered pattern may be varied without departing from the scope and spirit of the present invention. Alternative ducting patterns may be used which may assist in minimizing temperature differential effects as may be contemplated by those of ordinary skill in the art.

In the current embodiment, a muffler 62 connects to a reaction chamber exhaust 49, which is located on the end of the sample chamber opposite of the connection to the vortex tube with the mixing chamber 42. This muffler 62 exhausts the hot gas escaping from the reaction chamber 40. The reaction chamber muffler 62 is similar in all respects to the mufflers described previously. The reaction chamber muffler provides noise dampening characteristics that may be beneficial in decreasing the noise produced by the thermal cycling apparatus 10 from escaping gas being exhausted from the reaction chamber.

The configuration of the reaction chamber 40 may be varied to accommodate the needs of a user or manufacturer. For instance, the overall dimensions of the housing may be increased or decreased. Alternatively, the dimensions of the vortex flow conditioner and the sample chamber may be increased or decreased relative to one another or the overall dimensions of the reaction chamber. It is to be understood that the walls comprising the housing of the reaction chamber have a thickness. The thickness may vary to accommodate various additional features that may be incorporated into the reaction chamber, such as an optical detection system described below.

Optical Detection System

Real-time detection is conducted by monitoring the fluorescence in one of the capillaries 70 as the reaction progresses. The optical reading is taken once per cycle during the heat-to-denaturation step at a specified read temperature. Ideally, the optical measurements will be taken at a temperature after elongation is completed and primer-dimer artifacts have separated, but before newly formed target DNA denatures.

When the capillary 70 reaches the user designated temperature, a blue light source (LS450, Ocean Optics, Dunedin, FL) shines light at 470 nm into the capillary 70 where it reacts with the dye/double-stranded DNA complexes. When bound to double-stranded DNA, the SYBR Green dye emits light at a wavelength of approximately 520 nm. This optical signal is recorded by a CCD-array based spectrometer (USB2000, Ocean Optics). Information from the spectrometer is converted to give intensity vs. wavelength data. The user can use this data directly to plot the intensity for the whole spectrum or select a specific wavelength for a plot of intensity vs. cycle number. The sensitivity of the optical measurements can be adjusted according to the user specified integration time, which is the duration in which the spectrometer collects intensity data.

The versatility of using a CCD-array based spectrometer allows the use of other dye systems besides SYBR Green as described above. There are currently a number of other systems available that would emit signals at a different wavelength than 520 nm. The thermal cycling apparatus 10 of the present invention remains compatible with these systems since the CCD array operates over a wide range of wavelengths, approximately 375 to 1050 nm. The excitation source is also variable from the blue source (~470 nm) used in the thermal cycling apparatus 10 with the use of a different LED bulb.

The physical setup for an exemplary embodiment of the optical detection system 90 is shown in FIG. 13. A bifurcated fiber-optic cable 92 is used to transmit light between a single sample capillary 70′ and both the LED excitation source 94 and spectrometer 96, separately. The common end of the fiber enclosure sits directly below the sample capillary 70′ creating a union for signal transmission.

In the embodiment shown in FIG. 14, the thermal cycling apparatus 10 employs an alternate optical detection system 100 for providing on-line detection of DNA. In conjunction with the use of reporter dyes like SYBR Green, a light source, such as a blue light emitting diode (LED), is used in combination with a photodiode array including photoreceptor devices, such as a silicon filter/photodiodes, for amplification/detection of DNA in time scales of less than ten minutes. This time scale provides substantially real-time detection, which is an advantage over the prior art that typically employs standard gel agarose practices for the detection of the DNA, which take longer periods of time.

In the current embodiment of FIG. 14, the reaction chamber is shown to include a first LED 102, a second LED 104, and a third LED 106. Each LED is positioned opposite a corresponding first silicon filter/photodiode 112, a second first silicon filter/photodiode 114, and a third first silicon filter/photodiode 116, respectively. The optical detection system employs components that may be required to withstand approximately seventy to ninety psi. It is contemplated that the components of the optical detection system are placed behind a "sight window" and recessed into the sides of the reaction chamber 44. The sight windows may be plastic, glass, or other material, which allows for the passage of light from the LEDs and the receiving of light by the first silicon filter/photodiode. Each light emitting diode (LED) includes an emission filter (cutoff <470 nm) and a collimator. The light from each LED is sent through the sample and then received by the corresponding silicon filter/photodiode, which is positioned in correlation with the LED. The silicon filter is an absorption filter (cutoff >520 nm), which is bonded to the photodiode.

It is contemplated that the optical detection system may be variously configured. For instance, the light source may be a single LED. The single light source may emit a beam of light that contacts a mirror, which in turn directs the light through a sample and may be detected by a photo receptor device of a photodiode array. The mirror may be adjusted to direct the light through numerous samples. The number of LEDs, mirrors, and photoreceptor devices of a photodiode array employed with the present invention may vary to accommodate the thermal cycling apparatus 10 configuration.

Supplemental Heating

As shown in FIG. 15, a heating device 120 may be connected with the long "hot" end 24 of the vortex tube 20. The heating device 120 increases the temperature of the hot gas exiting the hot end 24 of the vortex tube 20. Since the thermal cycling apparatus 10 is currently rate-limited by its heating processes, a supplemental heating device has dramatic performance effects. It significantly reduces the impact a gas supply has on performance by allowing the device to be operable at lower pressures and flow rates. FIG. 15 shows the heating device as a heating coil that wraps around the hot end 24 of the vortex tube 20. It is contemplated that various other heating devices may be employed without departing from the scope and spirit of the present invention. For instance, the hot end of the vortex

tube may be at least partially surrounded by a heating block for delivering external heat. Also, an inline heater could be used to increase the temperature of the hot exit stream after it has exited the vortex tube.

The present invention further provides a method for performing a PCR process upon a DNA sample. In a first step a sample is prepared. The sample may be a standard PCR sample composed of a DNA+Primer+Polymerase mixture. It is to be understood that sample preparation may be changed as contemplated by those of ordinary skill in the art. In a second step a sample container for storing the sample is selected. The sample container may be a standard cuvette or various other devices. The number of sample containers selected may vary depending on the selection made in step three described below. In a third step the sample containers, including the samples, are placed within a sample housing 44 of the thermal cycling apparatus 10. In a preferred embodiment, the thermal cycling apparatus 10 is that shown and described in FIGS. 6 through 13. In alternative embodiments, the thermal cycling apparatus 10 is that shown and described in FIGS. 14 and 15. In a fourth step, a gas is selected for use with the thermal cycling apparatus 10. The gas may be a variety of gases, such as helium, nitrogen, oxygen, and the like, which have favorable heat transfer and thermal conductivity characteristics. Additionally, the gas may be stored and/or delivered at various pressures, such as atmospheric or above, to optimize the performance of the thermal cycling apparatus 10. In a fifth step the PCR process is carried out by forcing the gas through the thermal cycling apparatus 10 and the transfer of heat from the gas to the samples within the sample containers.

In an additional step, a user of the thermal cycling apparatus 10 may program, via the control logic employed with the thermal cycling apparatus 10, the valving system 30 to a pre-determined routine. For instance, the use of a helium gas may have a first valving routine for the performance of the PCR process. Alternatively, the use of Oxygen and/or Nitrogen gas may have alternative valve control routines for the performance of the PCR process. As described previously, the valving system is controlled electronically, therefore, in a preferred embodiment the valving routines are pre-programmed into the electronic control system and executed upon command. Alternatively, the valving system may be set manually by the user of the thermal cycling apparatus 10.

In a still further additional step, DNA detection may be performed through the use of an optical detection system. The optical detection system employed may be similar to one of the designs described previously or be of an alternative design. The DNA detection provides a real-time identification of DNA contained within the samples of the thermal cycling apparatus 10.

The present invention thus provides a mobile thermal cycling device for use at multiple locations and point-of-care sites. Mobility may increase the usefulness of the present invention as compared to past and current thermal cycling devices, by allowing the present invention to provide on-site PCR. Further, the present invention provides real-time detection of DNA amplified by the PCR process. In conjunction with the mobility of the present invention, this will provide an on-site method of performing the PCR process but also detecting the results of the process.

It is understood that the specific order or hierarchy of steps in the methods disclosed are examples of exemplary approaches. Based upon design preferences, it is understood that the specific order or hierarchy of steps in the method can be rearranged while remaining within the scope and spirit of the present invention. The accompanying claims present elements of the various steps in a sample order, and are not necessarily meant to be limited to the specific order or hierarchy presented.

EXAMPLE: PCR AMPLIFICATION OF 96BP Λ-DNA AMPLICON

By way of example, a rapid PCR process including Real-Time detection is performed utilizing the thermal cycling apparatus 10 of the present invention. The sensitivity of the thermal cycling apparatus 10 and its ability to monitor a PCR reaction real-time is demonstrated using a 96 bp λ-DNA amplicon. The experiment consists of varying the initial DNA concentrations from 0.25 to 100 fg, which corresponds to about 5 to 2000 copies. Reaction progress is monitored by measuring the fluorescence emitted at 520 nm by SYBR Green bound to double-stranded DNA complexes upon excitation at 470 nm. This dye binds to all double-stranded DNA and cannot distinguish between a specific PCR amplicon and primer-dimer artifact. Therefore, careful design of primers, optimum reaction conditions, and a Hot-Start PCR technique is very crucial when using SYBR Green for Real-Time PCR.

The oligonucleotide primers described by Braun, D., Goddard, N. L., and Libchaber, A., 2003, "*Exponential DNA Replication by Laminar Convection*," Physical Review Letters, 91, 158103, which is herein incorporated by reference in its entirety, were used to amplify the target λ-DNA fragment. The Primer sequences were 5'-GATGAGTTCGTGTCCGTACAACTGG -3' and 5'-GGGCAATCAGTTCATCTTTCGTCATGG-3' with melting temperatures of approximately 61°C and 62°C, respectively. The reaction mix (20 µl) consisted of 600 nanomolar of each primer, 0.25 fg to 100 fg of λ-DNA (Fermentas Inc., Hanover, MD), 5 mM Magnesium Sulfate, 200 µM dNTP's, 1X SYBR Green I (S7563, Molecular Probes), 600 µg/mL BSA, and 0.8 unit of KOD Hot Start Polymerase (Toyobo Co., Japan) in 1X KOD Hot Start Polymerase Buffer.

The speed of the thermal cycling apparatus 10 of the present invention is best utilized in combination with a fast enzyme, such as KOD Hot Start Polymerase. This enzyme requires an initial 30 second hot start exceeding 90°C to become fully activated. During this period, thermolabile antibodies bound to the DNA polymerase are inactivated, as described by Mizuguchi, H., Nakatsuji, M., Fujiwara, S., Takagi, M., and Imanaka, T., 1999, "*Characterization and Application to Hot Start PCR of Neutralizing Monoclonal Antibodies Against KOD DNA Polymerase*," J. Biochemistry, 126, pp. 762-768, which is herein incorporated by reference in its entirety.

Therefore, each reaction consisted of initial activation of the enzyme at 95°C for 30 seconds, followed by 45 cycles of [0 sec 95°C, 0 sec 63°C, 0 sec 72°C]. There are no holding times required for the denaturation and annealing steps since they happen almost instantaneously. Elongation occurs within a temperature range surrounding 72°C. Therefore, a large portion of the ramp time from the annealing to the denaturation temperature is utilized for elongation. If a fast enzyme such as KOD polymerase is used (approximately 300 nt/sec at 72°C), it is possible to copy up to 1,000 bp during the heating stroke, without pausing at 72°C. The amplification consisted of 45 cycles to demonstrate machine sensitivity to 0.25 fg of bacteriophage λ-DNA (~5 copies). Photon counting was carried out once per cycle using a 500 msec integration time when the sample reaches 80°C while heating to the denaturation temperature. Samples were also analyzed by 2.5% agarose gel electrophoresis in TAE buffer. The DNA fragments were visualized using 312 nm UV light and photographed with Polaroid Type 667 film.

The performance of the thermal cycling apparatus 10 is demonstrated using a 96 bp Lambda DNA amplicon. The sensitivity of the machine and the ability to monitor a PCR reaction real-time was tested by varying the initial DNA concentrations from 0.25 to 100 fg. Each reaction consisted of a hot start to activate the polymerase followed by 45 cycles between the denaturation, annealing, and elongation temperatures (95°C, 62°C, and 72°C, respectively) and had a total reaction time less than 12 minutes. FIG. 16 shows the temperature vs. time profile for the hot start and initial 10 cycles of the PCR reaction.

FIG. 17 shows that positive results were obtained for each initial concentration of λ-DNA while the negative controls (no initial λ-DNA) remained negative. This gel picture was used to conduct amplification and efficiency calculations to demonstrate machine sensitivity. An initial DNA amount of 0.25 fg produced an amplification of 2.7×10^{11}-fold, which corresponds to an overall efficiency of 79.5%.

The corresponding Real-Time PCR spectrometer data is shown in FIG. 18. The PCR reaction is deemed successful when the intensity increases beyond an established threshold value defined as 150% of the baseline intensity. The number of cycles necessary to reach threshold increases as the initial DNA concentration is reduced and in the absence of primer-dimer artifacts, the negative control should not exceed the threshold value. Quantitative PCR is possible by calculating the initial concentration of DNA using the number of cycles necessary for the intensity to surpass the threshold value. The 0.25 fg DNA sample surpassed threshold 8 minutes into the reaction at cycle number 34.

Successful PCR results are not limited to the above example. FIG. 19 demonstrates the thermal cycling apparatus's capability to successfully perform PCR for a wide range of DNA sources and amplicon sizes. The DNA sources used to date include human, bacteria, viral, and plasmid DNA. Within each category, several amplicons of sizes ranging from 96 bp to 7,800 bp were successfully PCR amplified. The times typically increase with amplicon size since larger amplicons can require longer holds at the denaturation, annealing, and most notably, the elongation temperatures. A single exception to this trend is evident from the HBV18 amplification. Its protocol is taken directly from a kit designed around a specific optical detection/dye system associated with it. Regardless, the thermal cycling apparatus 10 is a fully functional PCR machine capable of amplifying specific DNA target sequences in less time than conventional PCR devices.

It is believed that the present invention and many of its attendant advantages will be understood by the foregoing description. It is also believed that it will be apparent that various changes may be made in the form, construction and arrangement of the components thereof without departing from the scope and spirit of the invention or without sacrificing all of its material advantages. The form herein before described being merely an explanatory embodiment thereof. It is the intention of the following claims to encompass and include such changes.

CLAIMS

1. An apparatus for polymerase chain reaction amplification of one or more nucleic acid samples, each nucleic acid sample being held in a capillary, the apparatus comprising: a vortex tube comprising an inlet connected to a source of pressurized gas, a hot gas outlet and a cold gas outlet; and a reaction chamber for holding the capillary, the reaction chamber being in fluid communication with the hot gas outlet and the cold gas outlet of the vortex tube to receive gas streams therefrom.

2. The apparatus of claim 1 wherein the apparatus further comprises valving means for selectively controlling the temperature of the gas stream in the reaction chamber.

3. The apparatus of claim 2 wherein the apparatus further comprises a cold gas conduit connected at one end to the cold gas outlet of the vortex tube and at its other end to both a cold flow control valve in communication with the reaction chamber and at least one exhaust valve for exhausting gas from the cold gas conduit.

4. The apparatus of claim 1 wherein the apparatus further comprises a controller for controlling the temperature of the gas in the reaction chamber.

5. The apparatus of claim 3 wherein the reaction chamber comprises a mixing chamber and a sample chamber, the mixing chamber being in fluid communication with the hot gas outlet through a first mixing chamber inlet and the cold gas control valve through a second mixing chamber inlet.

6. The apparatus of claim 5 wherein the mixing chamber is generally cylindrically shaped and wherein the first and second mixing chamber inlets are substantially perpendicularly oriented.

7. The apparatus of claim 6 wherein the second mixing chamber inlet is further configured to direct the cold gas stream tangentially to the cylindrically shaped mixing chamber.

8. The apparatus of claim 5 wherein the reaction chamber comprises at least one duct operably configured to receive a capillary and a sample chamber conduit to direct the gas from the mixing chamber to the at least one duct.

9. The apparatus of claim 8 wherein the sample chamber conduit comprises a pair of walls tapering inwardly from an entrance to an exhaust to compensate for heat loss.

10. The apparatus of claim 8 wherein the apparatus comprises more than one duct and wherein the ducts are arranged in a staggered configuration.

11. The apparatus of claim 8 wherein the apparatus further comprises a duct for receiving a thermocouple and wherein the apparatus further includes a processor connected to the thermocouple for monitoring and controlling the temperature in the reaction chamber.

12. The apparatus of claim 1 wherein the apparatus further comprises an optical detection system for real-time analysis of a sample at a pre-determined read temperature.

13. The apparatus of claim 13 wherein the optical detection system comprises a bifurcated fiber-optic cable used to transmit light between a single sample capillary and both an LED excitation source and a spectrometer, the light reacting with the sample held in the capillary and producing a signal, the signal being recorded by the spectrometer.

14. The apparatus of claim 13 wherein the optical detection system comprises one or more light emitting diodes each having an emission filter and a collimator, and one or more corresponding silicon filters bonded to one or more photodiodes, the light from each light emitting diode being sent through the sample and received by the corresponding silicon filter and photodiode, which is positioned in correlation with the LED.

15. The apparatus of claim 1 wherein the hot gas outlet of the vortex tube further comprises insulation to reduce heat loss.

16. The apparatus of claim 1 wherein the hot gas outlet further comprises a supplemental heating apparatus to increase the temperature of the hot gas exiting the hot gas outlet of the vortex tube.

17. The apparatus of claim 16 wherein the supplemental heating apparatus comprises a heating coil.

18. The apparatus of claim 16 wherein the supplemental heating apparatus comprises a heating block.

19. A thermal cycling apparatus for subjecting one or more samples to rapid thermal cycling, the apparatus comprising: a source of pressurized gas, a vortex tube connected to the source of pressurized gas and providing hot and cold gas streams to provide the medium for convective heat transfer, a sample chamber operably configured to hold one or more sample cuvettes, and a valve system to control the gas flow to cycle the samples through the desired sample temperature change.

20. The thermal cycling apparatus of claim 19 wherein the hot gas stream is in the range of approximately 25°C to 200°C and the cold gas stream is in the range of approximately -50°C and 25°C.

21. The thermal cycling apparatus of claim 19 wherein the samples are used for polymerase chain reaction and comprise DNA, a DNA polymerase, oligonucleotide primers, and deoxynucleotide precursors.

22. The thermal cycling apparatus of claim 19, wherein the inlet pressure to the vortex tube system is between atmospheric pressure and 250 psi.

23. The thermal cycling apparatus of claim 19, wherein the sample chamber includes a chamber flow conduit and the chamber flow conduit has cross-sectional area not exceeding 4 in^2.

24. The thermal cycling apparatus of claim 19, wherein the sample cuvettes are glass capillaries with an outer diameter in the range of 0.5 mm to 5 mm.

25. The thermal cycling apparatus of claim 23, wherein the hot gas velocity within the chamber flow conduit exceeds 5 m/s and wherein the cold gas velocity within the chamber flow conduit exceeds 5 m/s.

26. The thermal cycling apparatus of claim 19 further comprising a means for feedback control, the means for feedback control being a thermal sensor and a central processing unit.

27. The thermal cycling apparatus of claim 19, wherein the pressure inside the sample chamber is between approximately atmospheric pressure.

28. The thermal cycling apparatus of claim 19, wherein the source of pressurized gas is compressed air.

29. The thermal cycling apparatus of claim 19, wherein the gas for the source of pressurized gas is selected from the group consisting of helium, nitrogen, hydrogen, neon, methane, argon, krypton, carbon dioxide, and mixtures thereof.

30. The thermal cycling apparatus of claim 19, wherein the hot gas stream and cold gas stream are mixed in a mixing chamber before entering the sample chamber.

31. The thermal cycling apparatus of claim 19, wherein only the cold gas stream is directed using electronically-actuated valves.

32. The thermal cycling apparatus of claim 19 further comprising a fluorescence based detection system to provide real-time detection for one or more samples.

33. The thermal cycling apparatus of claim 19, wherein the temperature of samples is rapidly and controllably increased and decreased at a rate of at least as great as 3°C/second.

34. The thermal cycling apparatus of claim 19, wherein the sample cuvette arrangement is staggered at an angle within the range of 0 to 5 degrees per side.

35. The thermal cycling apparatus of claim 19 further comprising exhaust mufflers to reduce operating noise.

36. The thermal cycling apparatus of claim 20, wherein the hot gas outlet of the vortex tube is insulated to prevent heat loss.

37. The thermal cycling apparatus of claim 20, wherein the hot gas stream exiting the hot gas outlet of the vortex tube is directed using electronically actuated valves.

38. A polymerase chain reaction method, comprising the steps of:

A. providing a vortex tube having an inlet connected to a source of pressurized gas, a hot gas outlet and a cold gas outlet, the vortex tube providing a hot gas stream and a cold gas stream;

B. providing a reaction chamber for holding a nucleic acid sample, the reaction chamber being in fluid communication with the hot gas outlet for receiving the hot gas stream and the cold gas outlet for receiving the cold gas stream;

C. providing a valving system on the cold gas outlet to control the amount of cold gas entering the reaction chamber; and

D. cycling the temperature in the reaction chamber through selective introduction of cold gas from the cold gas outlet of the vortex tube.

39. The method of claim 38, wherein the step of providing a vortex tube further includes the hot gas stream produced by the vortex tube exiting the hot gas outlet with a temperature within the range between 25°C and 200°C and the cold gas stream exiting the cold gas outlet with a temperature within the range of -50°C and 25°C.

40. The method of claim 38, wherein the step of providing a vortex tube further includes providing a vortex tube having an inlet pressure to the vortex tube that is above atmospheric pressure.

41. The method of claim 38, further including the step of:

E. Selectively introducing the cold gas stream in the reaction chamber to provide substantially isothermal chamber conditions.

42. The method of claim 38 further including the step of providing a feedback control having a thermal sensor and a processor.

43. The method of claim 38, wherein the step of providing a reaction chamber for holding a nucleic acid sample includes holding a DNA/Primer/Polymerase sample and wherein the step cycling the temperature in the reaction chamber comprises cycling the sample between the denaturation, annealing and elongation temperatures of a polymerase chain reaction process.

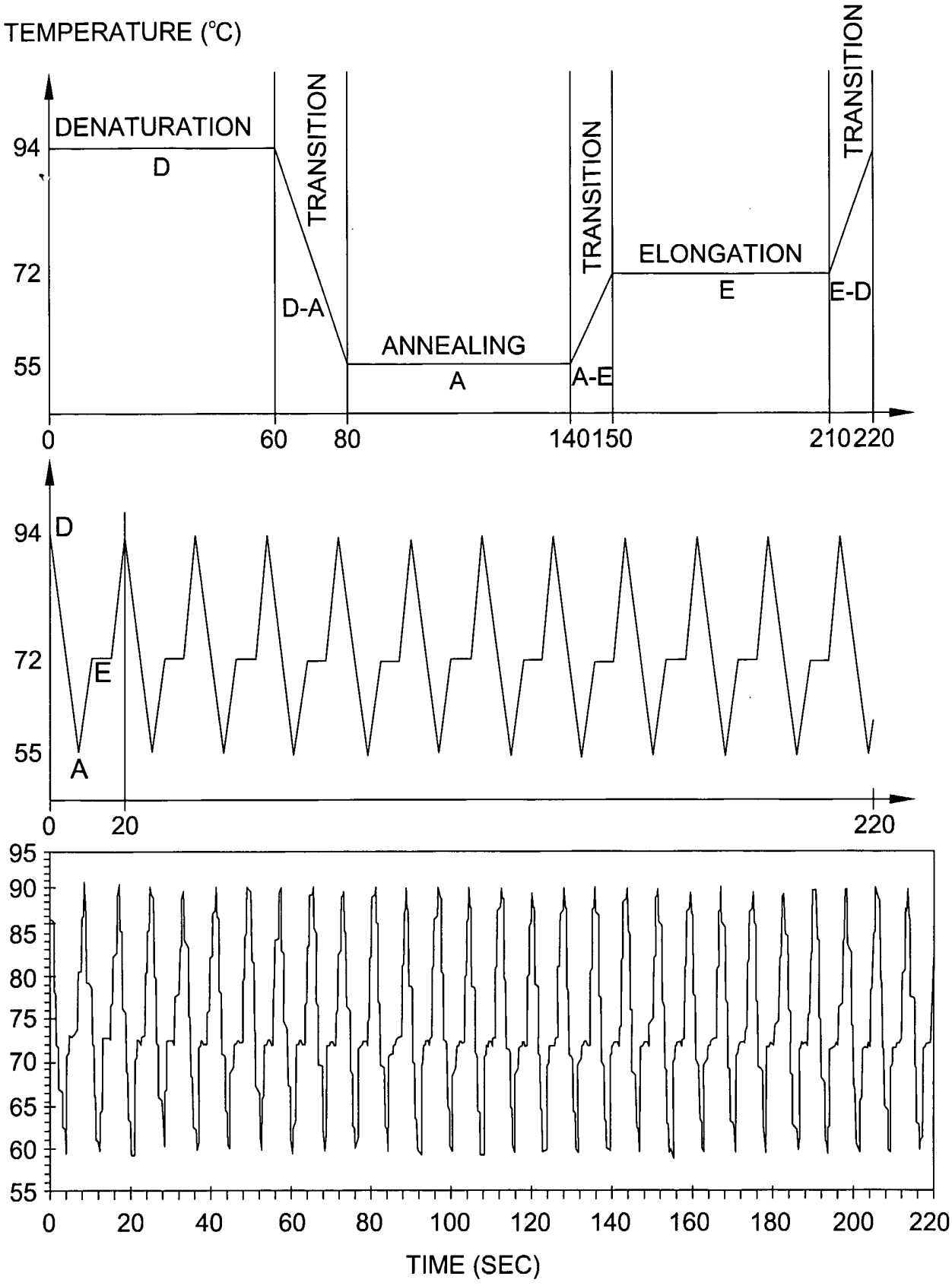
TEMPERATURE (°C)
DENATURATION
D
TRANSITION
D-A
ANNEALING
A
TRANSITION
A-E
ELONGATION
E
TRANSITION
E-D
94
72
55
0
60
80
140
150
210
220
D
E
A
20
95
90
85
80
75
70
65
60
TIME (SEC)
40
100
120
160
180
200

FIG. 1.

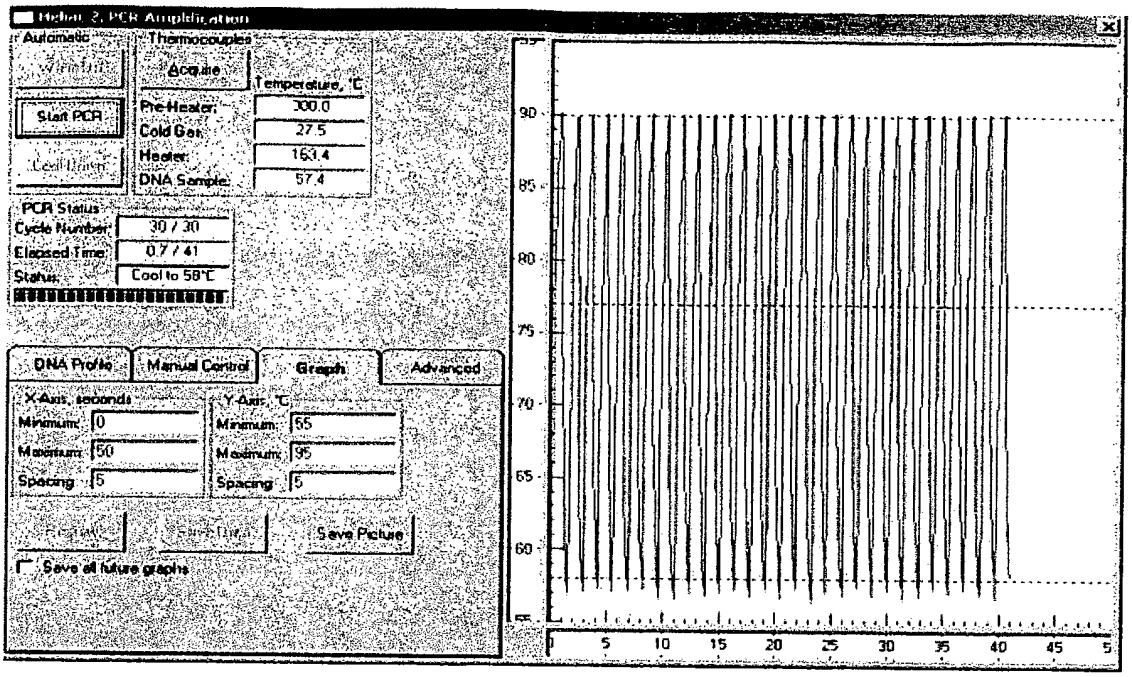
Automatic
Thermocouples
Acquire
Temperature, °C
Start PCR
Pre-Heater:
300.0
Cold Gas:
27.5
Heater:
163.4
DNA Sample:
57.4
PCR Status
Cycle Number:
30 / 30
Elapsed Time:
0.7 / 41
Status:
Cool to 58°C
DNA Profile
Manual Control
Graph
Advanced
X-Axis, seconds
Minimum:
0
Maximum:
50
Spacing:
5
Y-Axis, °C
Minimum:
55
Maximum:
95
Spacing:
5
Save Picture
Save all future graphs
90
85
80
75
70
65
60
0
5
10
15
20
25
30
35
40
45

FIG. 2

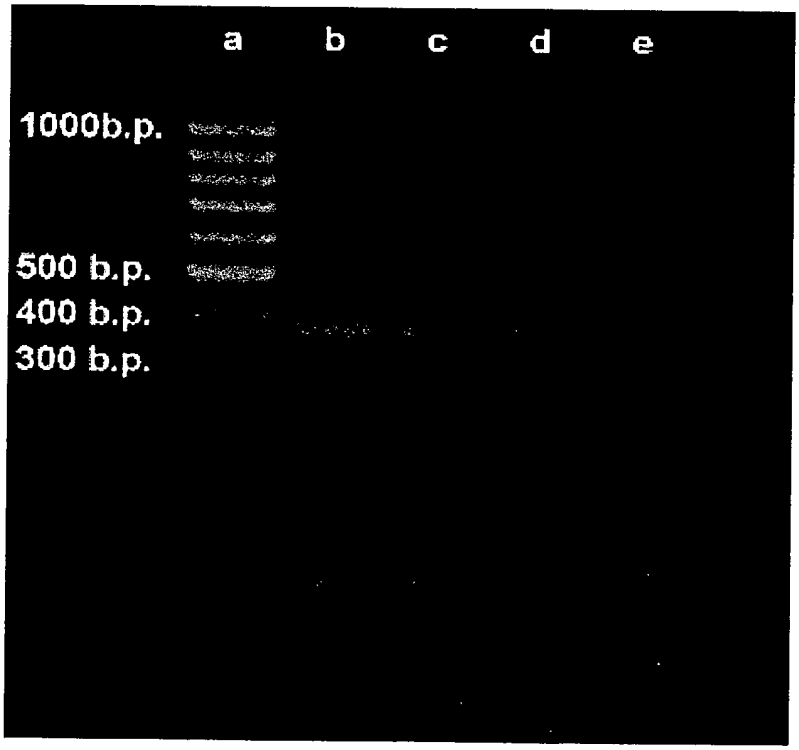
a
b
c
d
e
1000b.p.
500 b.p.
400 b.p.
300 b.p.

FIG. 3

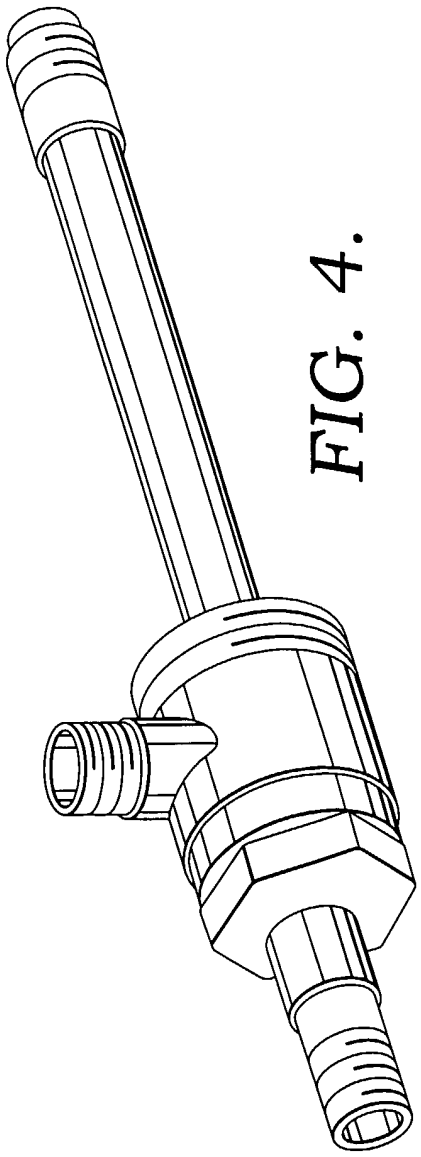

FIG. 4.

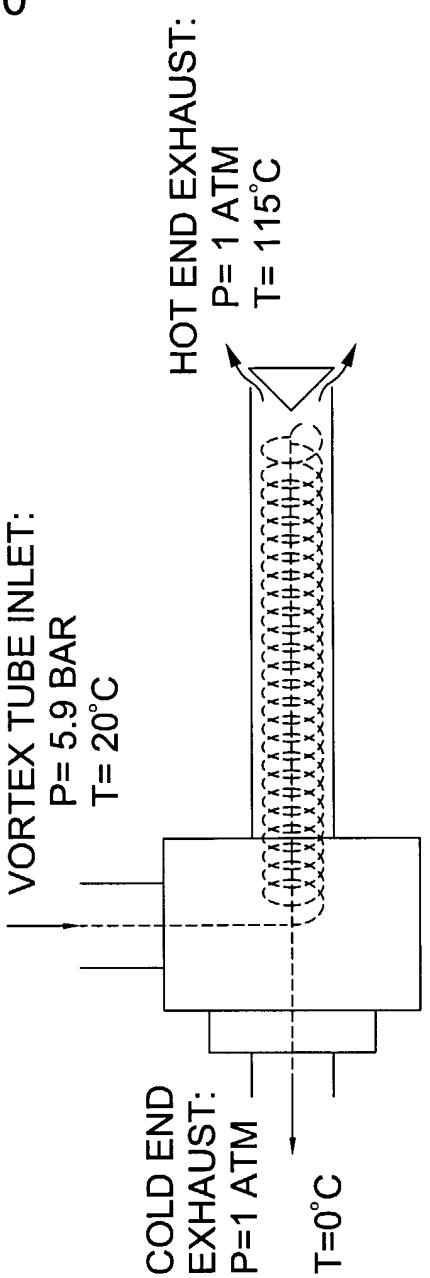
HOT END EXHAUST:
P= 1 ATM
T= 115°C
VORTEX TUBE INLET:
P= 5.9 BAR
T= 20°C
COLD END
EXHAUST:
P=1 ATM
T=0°C

FIG. 5.

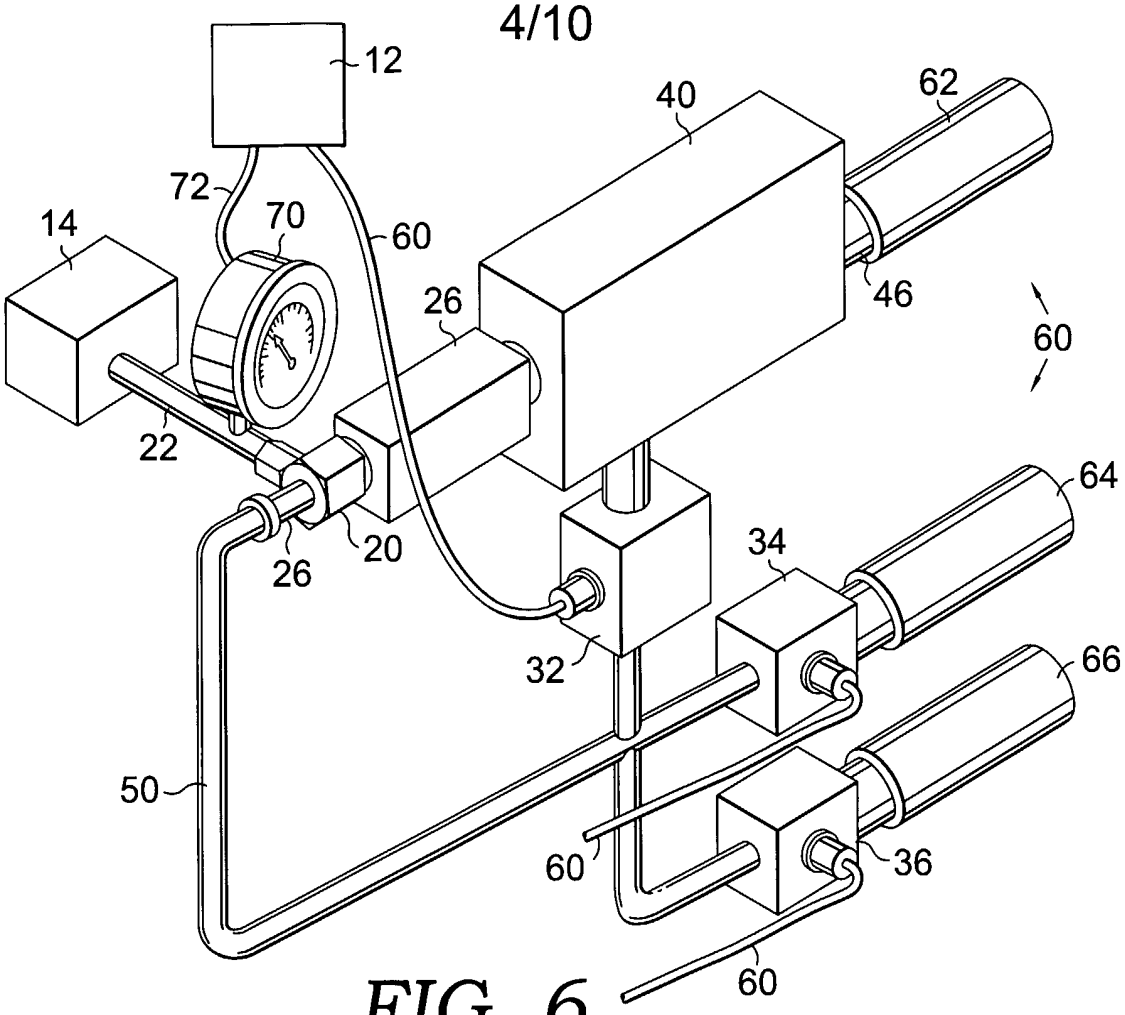
12
40
62
72
70
60
14
26
46
60
22
64
26
20
34
32
66
50
60
36
60

FIG. 6.

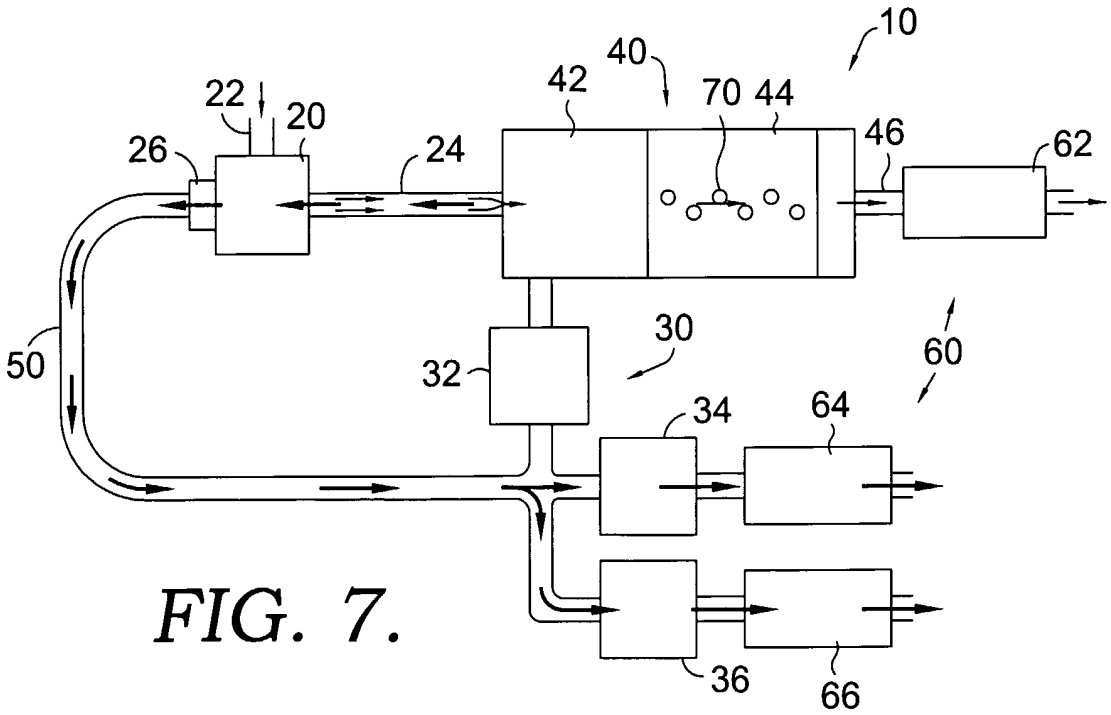
10
40
42
70
44
22
20
46
62
26
24
50
30
60
32
34
64
36
66

FIG. 7.

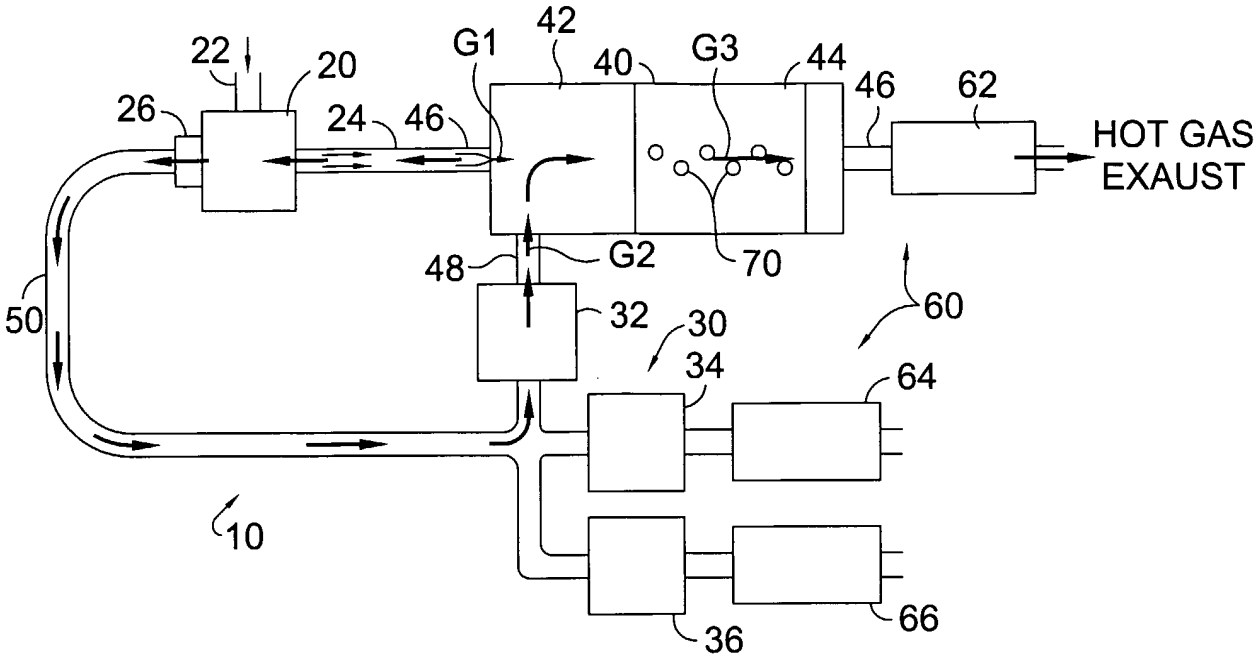
22
20
26
24
46
G1
42
40
G3
44
46
62
HOT GAS
EXAUST
48
G2
70
50
32
30
60
34
64
10
66
36

FIG. 8.

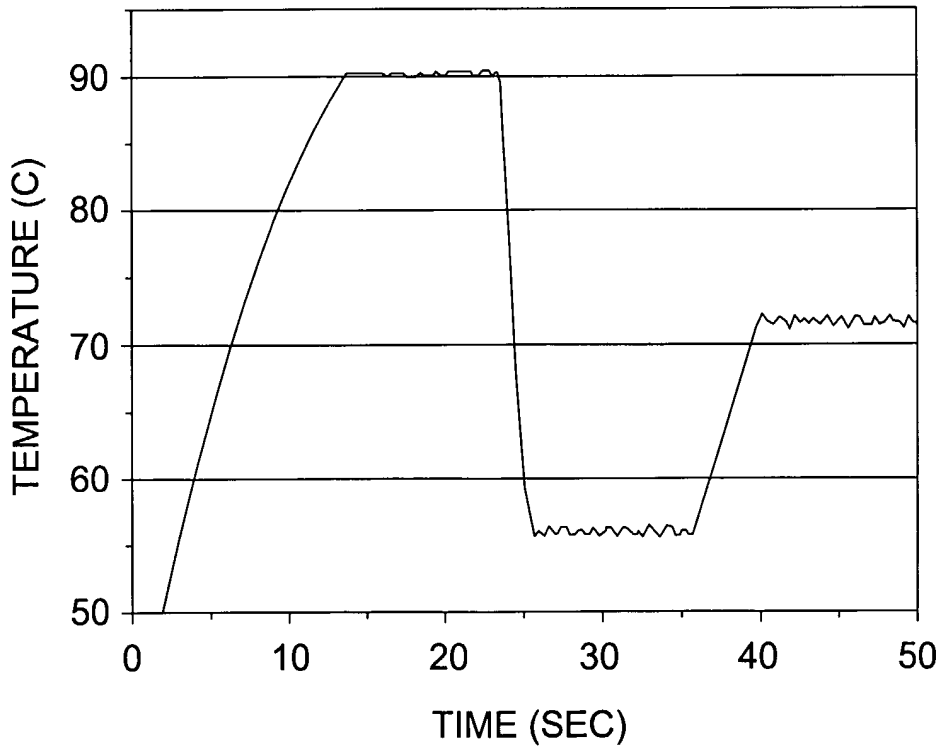
TEMPERATURE (C)
90
80
70
60
50
0
10
20
30
40
50
TIME (SEC)

FIG. 9.

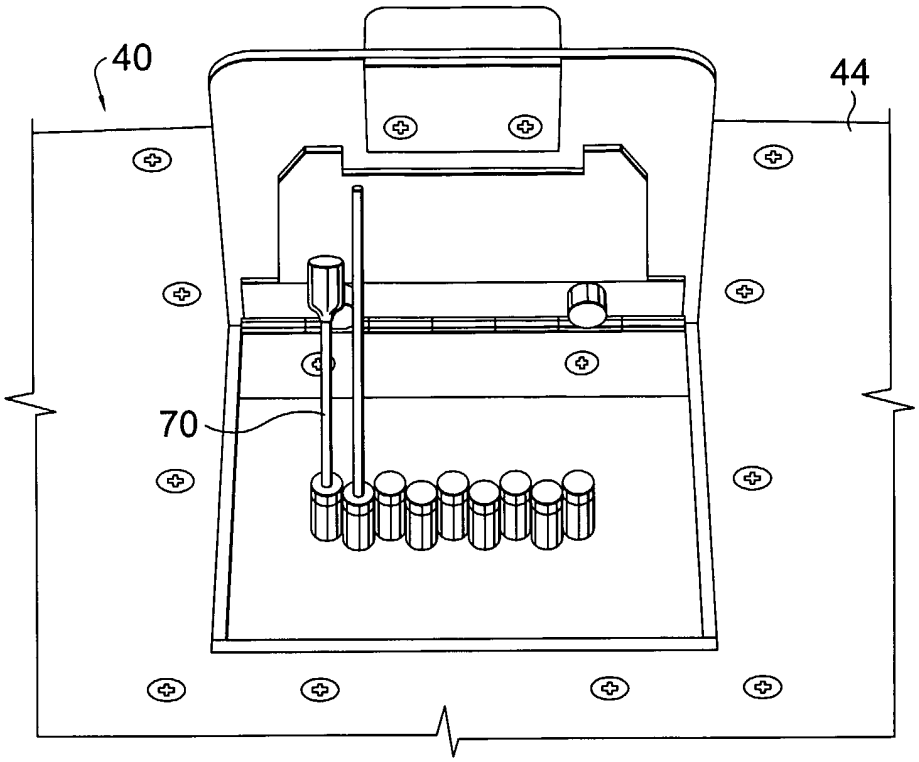

FIG. 10.

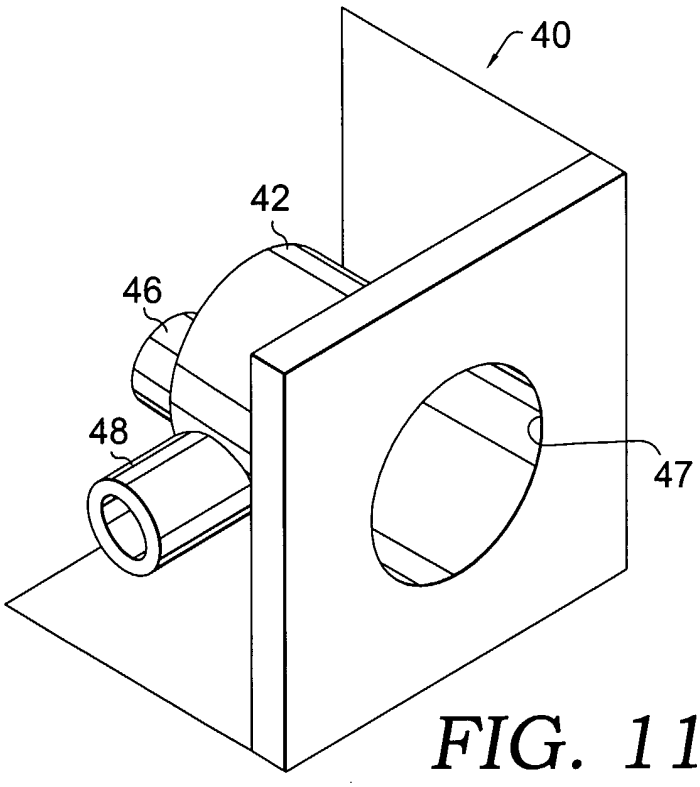

FIG. 11.

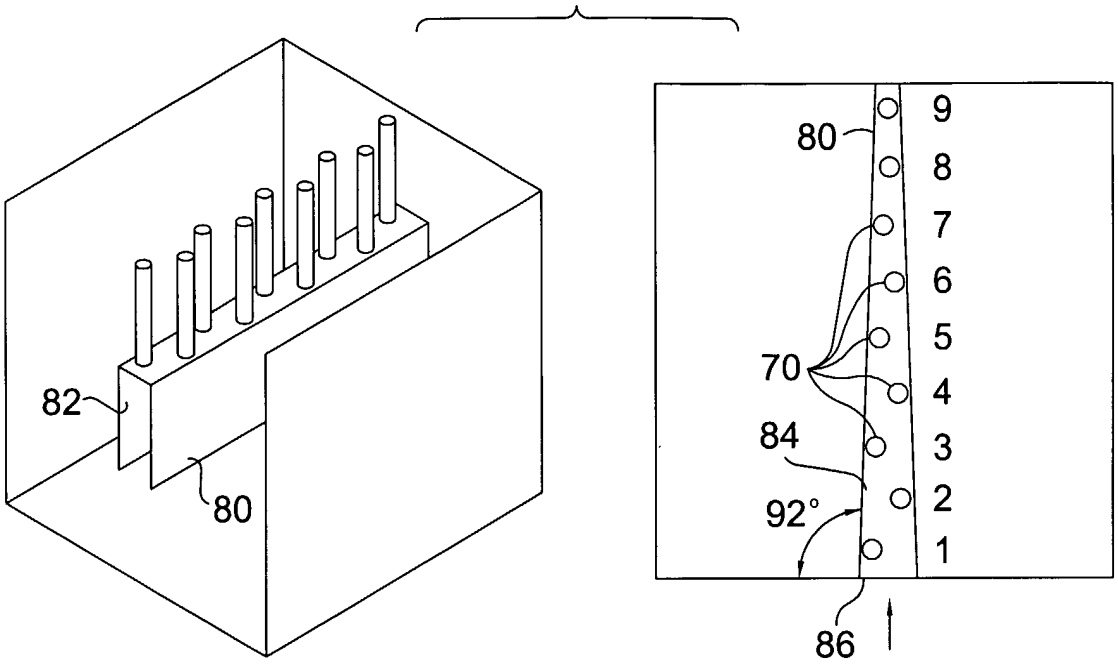

FIG. 12.

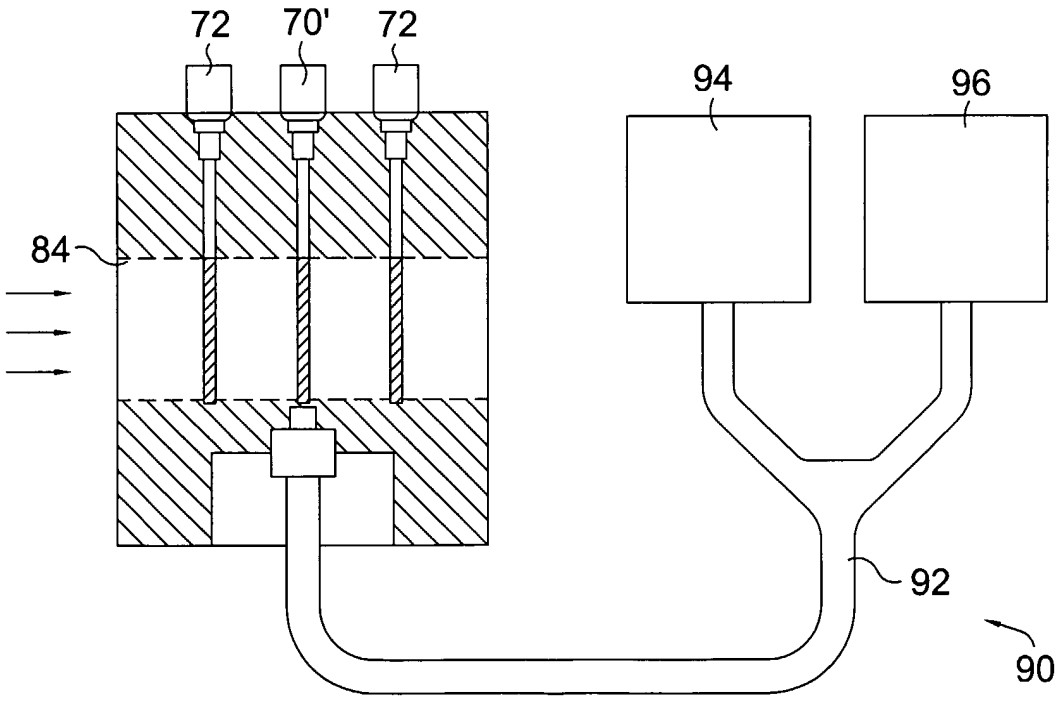

FIG. 13.

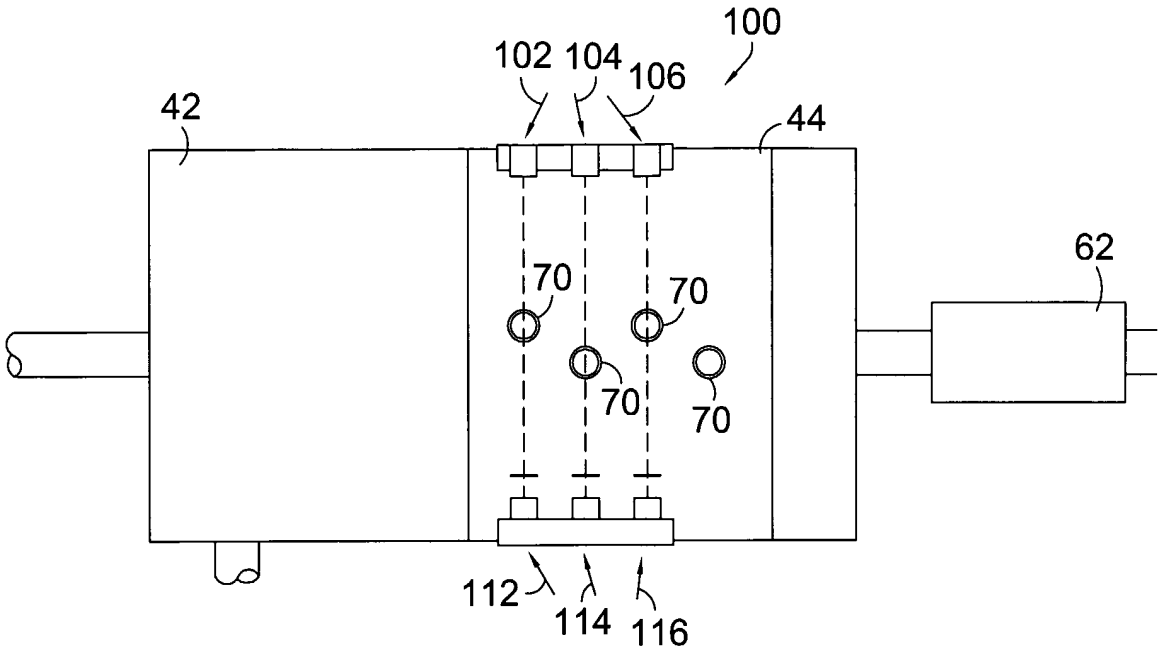
100
102
104
106
42
44
62
70
70
70
70
112
114
116

FIG. 14.

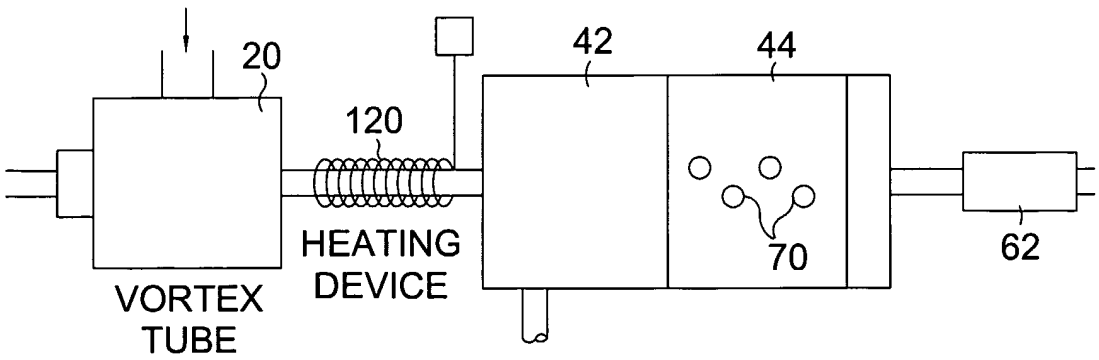
20
120
42
44
70
62
HEATING
DEVICE
VORTEX
TUBE

FIG. 15.

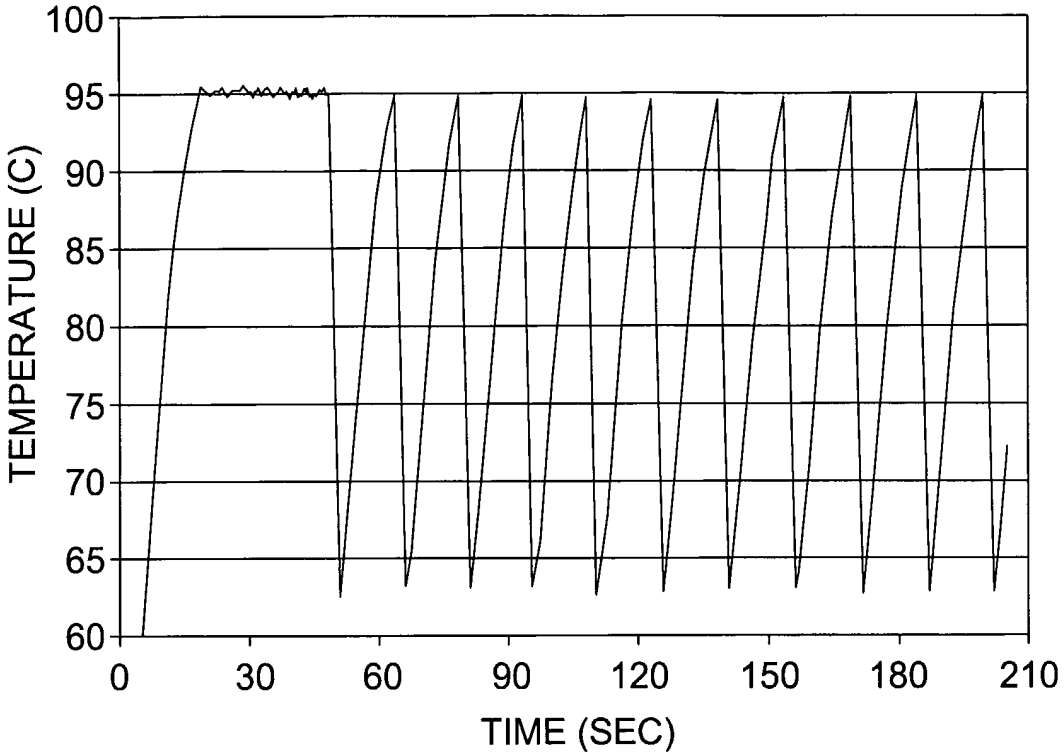
100
95
90
85
80
75
70
65
60
TEMPERATURE (C)
0
30
60
90
120
150
180
210
TIME (SEC)

FIG. 16.

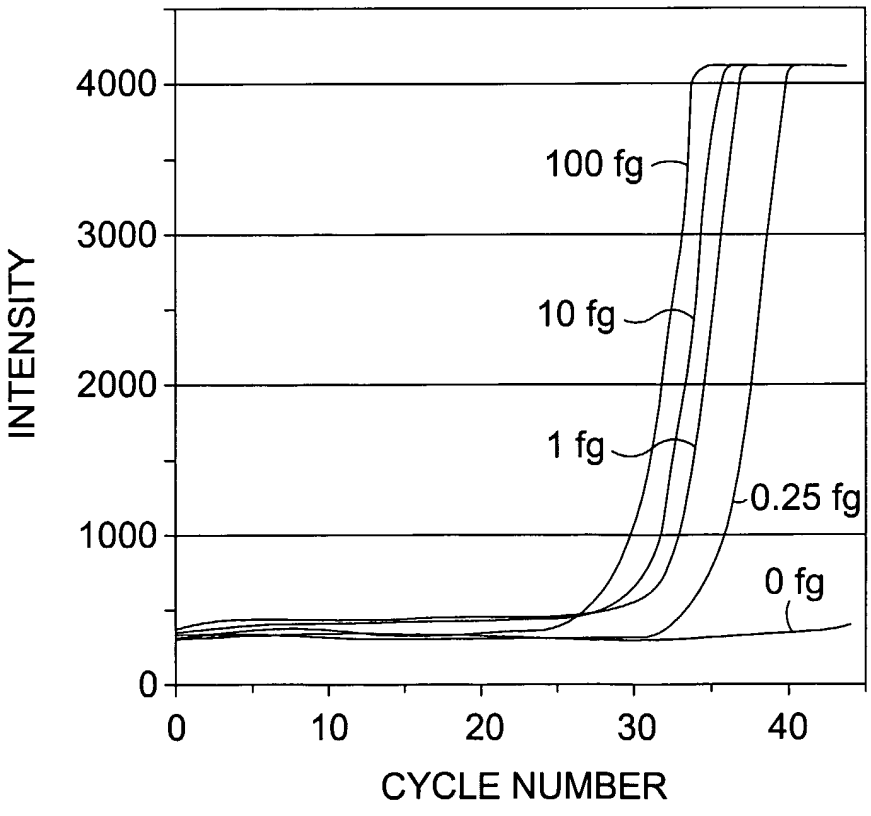
4000
3000
2000
1000
0
INTENSITY
100 fg
10 fg
1 fg
0.25 fg
0 fg
0
10
20
30
40
CYCLE NUMBER

FIG. 18.

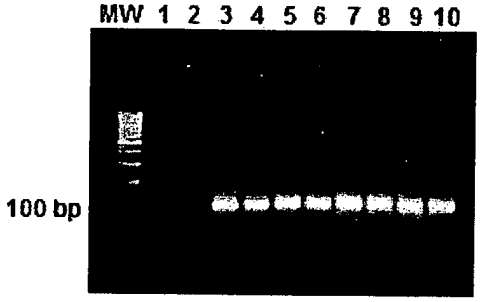

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/16739

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12P 19/34; C12M 1/38
US CL . 435/91.2, 286.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 91.2, 286.1, 286.6, 287.2, 288.7, 303.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,140,054 A (WITTWER et al.) 31 October 2000 (31.10.2000), see entire document.	1-43
Y	JP 4-180843 A (OOISHI) 29 June 1992 (29.06.1992), see entire document.	1-43
Y	US 6,448,066 B1 (WHEATCROFT) 10 September 2002 (10 09.2002), see entire document.	1-43

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search
17 October 2005 (17.10.2005)

Date of mailing of the international search report
10 NOV 2005

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